APR 25 1927
Medical Lib

Vol. XII APRIL, 1927 No. 7

The Journal of Laboratory and Clinical Medicine

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The Journal of Laboratory and Clinical Medicine

VOL. XII ST. LOUIS, MO., APRIL, 1927 NO. 7

CLINICAL AND EXPERIMENTAL

ON NEPHELOMETRY*

BY HANS KLEINMANN, M.D., PH.D., BERLIN, GERMANY

I. PREFACE

ADVANCES in physiologic chemistry are connected with methods allowing of estimating accurately and at the same time rapidly the strength of very dilute solutions of inorganic, as well as organic substances. For only by such methods is it possible to make series of experiments with biologic liquids, i.e., blood or serum, such as are necessary for the investigation of physiologic and pathologic processes.

During the last few years investigators have, therefore, striven to develop special analytic methods for biologic work. Optical methods have proved especially suitable and a large number of analytic processes based on colorimetric estimation have been described.

Besides these methods based on the estimation of the intensity of coloring, called forth in solutions by chemical reactions, an altogether independent branch of optical analysis has been developed, which is based on the comparing and gauging of the turbidity of solutions. It has therefore been given the name of nephelometry.

In colorimetric analysis the intensity of the coloring of a solution, produced by a certain reaction, is used to gauge the strength of the solution by comparing it with a solution containing the reacting substances in known quantity (a standard solution). In similar manner the strength of a solution is estimated nephelometrically, not, it is true, by means of a reaction producing color, but of a reaction producing turbidity, by comparing the turbidity produced in a solution of the same substance of known strength.

*From the Chemical Department of the Pathological Institute of the Charité, Berlin. Received for publication, October 23, 1926.

In recent years a series of papers on nephelometry has been published. Reactions producing turbidity have been described and apparatus designed by means of which turbid solutions could be compared and estimated, and much careful work has been devoted to ascertaining what relation exists between the turbidity produced and the quantity of the substance producing it and how that quantity may be calculated from the data obtained in gauging the turbidity.

The author has, by a series of investigations begun in 1919[1] and continued since then, shown that the relation between turbidity and concentration is very simple and that under certain working conditions turbidity and concentration are directly proportional. He succeeded in proving that nephelometry may be employed in the same manner and to the same purpose as the allied science of colorimetry.

This analogy between colorimetry and nephelometry is moreover completely in accordance with theoretical considerations on the nature of Tyndall-light, for it is this kind of light that is measured in nephelometric analysis.

The laws of nephelometry could not, however, be established on a firm basis, nor could nephelometric analysis be carried on successfully, unless an instrument, a nephelometer, could be designed, in which the optical defects of the hitherto known apparatus, the real cause of the discrepancies between the results of analytical work and the simple laws governing the phenomena, were avoided.

The author has designed an instrument of this kind and has, by means of it, succeeded in fully confirming by practical analysis the laws of nephelometry deduced from theoretical considerations.

With the help of this new type of nephelometer, a large number of nephelometric methods were either tested or newly developed. The author succeeded in finding and developing nephelometric methods suitable for biochemical analysis as well as for investigations on ferments, and applicable also to purely chemical work on colloidal substances.

The following pages contain a summary of the results of the work done in the last few years. The theory of nephelometry and its most important applications will be further discussed and the apparatus and methods described.

II. THEORY OF NEPHELOMETRY

Richards and Wells[2, 3] were the first to make a long series of quantitative nephelometric investigations. These authors, who worked with a primitive instrument of their own construction, state the maximum error in nephelometric analysis to be 5 per cent and that within this limit they had found turbidity and concentration to be proportional. Their work, however, shows how difficult accurate nephelometric measurements were at that time, how uncertain the results were, and how high the limit of possible error had to be drawn. Nephelometric analysis was subsequently developed further, mainly by Americans. Kober[4] as well as Bloor[5] designed instruments which by a simple manipulation could be changed from a Duboseq colorimeter into a nephelometer. All their investigations, however, seemed to show that turbidity and concentrations are not proportional. Even with solutions of a strength

nearly approaching that of their standard solution they state that they had found results incompatible with the principle of proportionality and their results finally led them to establish a complicated mathematical formula from which the concentration of the solution could be calculated after measurement, or determined graphically by means of a diagram. Their results and conclusions were, however, at variance with the theoretical considerations of Rayleigh on the intensity of the diffracted light emitted by a Tyndall-strip. According to Rayleigh the intensity of the diffracted light is

$$J = \frac{c \cdot v \cdot k}{y^2 \cdot s}$$

J being the intensity of the light, c the concentration, v the volume of the particles, s their specific weight, y the wave length of the light and k a constant.

Now in comparing two turbid solutions, the values v, k, y and s cancel each other and we therefore have

$$J : J_1 = c : c_1$$

or in words: *Provided the suspended particles are of equal size, the turbidity of the two solutions is proportional to their concentration.*

Nevertheless it was, of course, quite possible that some source of error caused the actual measurements to diverge more or less from this law.

Investigations, the particulars of which cannot be entered into here[1, 6, 19] and to which we must therefore refer the reader, showed, however, that the Duboscq colorimeter when used as a nephelometer, as was done by Kober and Bloor, possesses several optical defects to which the discrepancies between their results and the relation between turbidity and concentration, demanded by theory, are due.

The writer, therefore, aimed at designing an instrument in which the defects of the Duboscq colorimeter, when used as a nephelometer, would be avoided. With the assistance of the firm of Schmidt and Haensch, Berlin, he succeeded in constructing a new type of nephelometer fulfilling this condition.

The results obtained in working with this instrument soon showed that, certain conditions being duly observed, turbidity and concentration are accurately proportional. This law was confirmed again and again in a series of investigations carried out with a great variety of reactions producing turbidity.

The high accuracy of the measurements obtained with this new apparatus as well as its handiness and the simplicity of its design enables us to place nephelometry on a level with colorimetry as an analytical method.

The conditions which must be observed in nephelometric investigations will be specified in detail further on. Results showing the accuracy of measurement obtainable with the instrument will be given and its application to various kinds of analytical work discussed.

We will first give a description of the nephelometer, its modifications and its manipulation.

III. DESCRIPTION OF THE NEW TYPE OF NEPHELOMETER*

(a) *The Macronephelometer.*—The working principle of this instrument consists in producing Tyndall-cones (the height of which can be varied) in two turbid media arranged side by side and measuring and comparing their luminous intensity in a line perpendicular to their axis by means of suitable optical fittings. The only difference between this instrument and the instruments usually employed for colorimetric measurement, therefore, is that the light, the intensity of which is measured, is diffracted, not transmitted, light. Thus it is possible to apply the principle, familiar to us from colorimetric methods, of varying the luminous intensity in fixed proportions by varying the "height or thickness of the layer," in our case the diameter of the Tyndall-cones. The concentration of two turbid solutions should therefore,

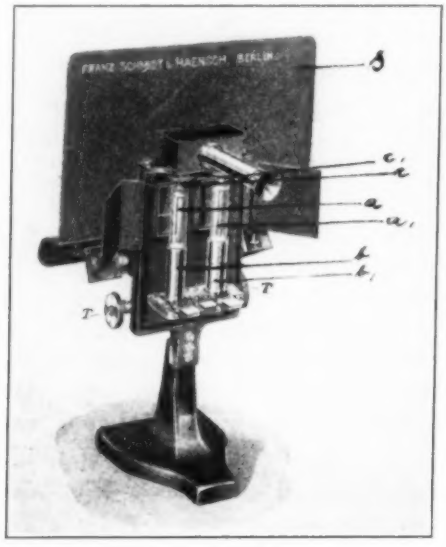

Fig. 1.

if the law of proportionality holds good, as in colorimetric measurements, be inversely proportional to the diameter of the Tyndall-cones produced in them, when reduced to equal luminous intensity. We must, however, keep in mind that in turbid media the intensity of the Tyndall-light increases with the concentration, owing to the increase in the number of the diffracting particles, whereas in colorimetric analysis the luminous intensity on the contrary decreases with concentration. From the nephelometers hitherto constructed on the same principle, that of the applied Duboscq colorimeter, the present instrument differs mainly in certain modifications by which the optical and technical defects peculiar to these instruments, the nature of which I have explained elsewhere, are avoided.

The new nephelometer is shown in the annexed illustrations of which

*This instrument as well as all accessories will be supplied by the firm of Schmidt & Haensch, Berlin, or by: American Kreuger & Toll Corporation, New York City, 114-118 Liberty Street.

Fig. 1 is an objective front view, Fig. 2 a diagrammatic sketch of the design in side elevation.

Fig. 1 and Fig. 2 show the two test tubes a and a_1 into which the standard solution and the solution to be tested are filled. They hold about 12 c.c. each. These test tubes are carried in metal casings b and b_1, in which they

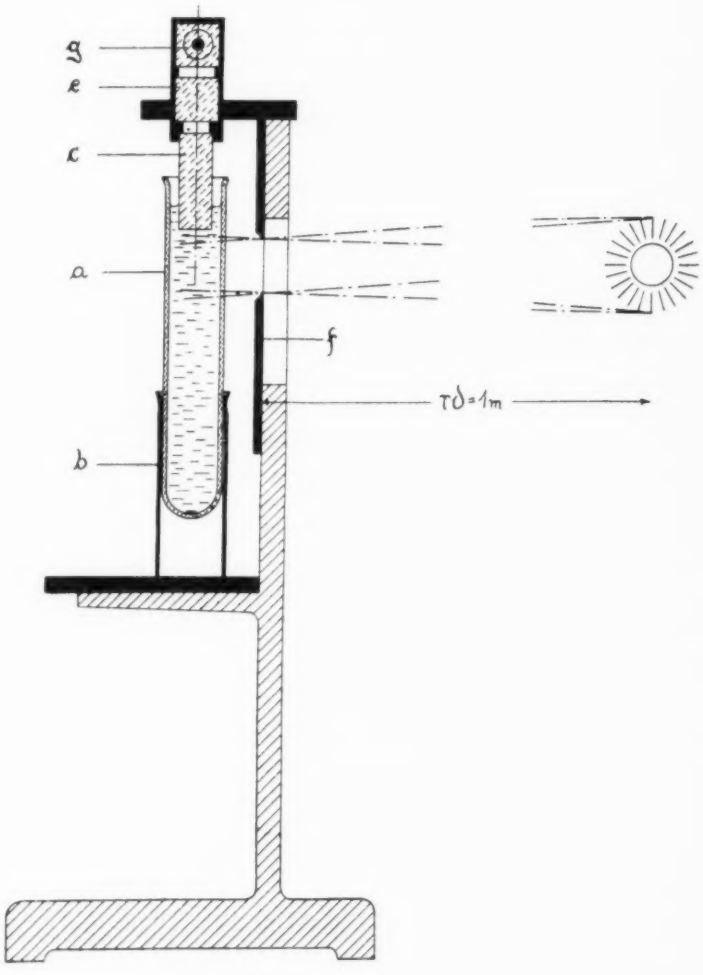

Fig. 2.

fit easily, so that they can be moved up and down without difficulty. The casings are fitted on spring bases adapted to slide in a suitable frame.

A beam of light is thrown on the test tubes by a lamp placed in front of the instrument and the Tyndall-cones thus produced are observed and gauged in a line perpendicular to the axis of the beam.

For this purpose the diffracted light is made to pass first through two solid glass cylinders c and c_1, identical in shape and size and cut out of adjacent parts of the same block of glass, in order to render their action on the light absolutely symmetrical. To eliminate the error which may be caused

by observing the surface of the liquid, the lower parts of the cylinders are immersed in it. By a suitable arrangement of diaphragms the cylinders receive light only from the central part of the Tyndall-cones.

The section of the tubes exposed to light and therefore also the diameter of the Tyndall-cones can be varied at will by varying the height of the windows f and f_1 through which the light reaches the turbid solutions. These windows are about 4.5 cm. high and about 2 cm. wide. The bottom part of the window openings is closed by a movable metal plate with a sharp edge, fitted on the interior surface of the wall of the instrument, so that the shadow limiting the illuminated section is very sharply defined. These metal shutters can be displaced by means of rack and pinion and the displacement read by means of verniers. The height of the windows can be varied independently for each Tyndall-cone, by means of the corresponding milled screw heads t and t_1, from complete closure to the full height of 4.5 cm.

The verniers may be conveniently read from the back, the observer's side, of the instrument in the prisms L and L_1, that receive light from the observation lamp and are adapted to be moved laterally to bring the scale into focus.

The scale is divided into millimeters and reads from zero (closed window) to 45 (window opened full). By means of the verniers the scale allows reading of 0.1 mm. The observer is screened from the light of the lamp by a removable screen s. In order to exclude the light reflected from surrounding objects the turbid solutions are enclosed in a box of blackened sheet-metal, not shown in the drawing, which is permanently fitted on the instrument and can be easily opened and closed.

A frosted Osram lamp of 100 candle power is preferably employed as source of light. It should be installed at a distance of 75 cm. in front of the instrument in a line with its optical axis and on a level with the windows.

(b) *The Micronephelometer.**—In working with material of which only small quantities are available, our instrument showed a disadvantage not present in the previously known form of nephelometer in which about 12 c.c. were required to fill the vessels used for measuring.

I, therefore, set about to design a modified form of his instrument which would allow the examination of smaller quantities of solution. In cooperation with the firm of Schmidt & Haensch I constructed a supplementary fitting to be used in the above-described nephelometer in place of the test tubes containing the solutions. By means of this modification the instrument may be used both as macronephelometer (taking 12 c.c. of solution) and as micronephelometer, taking smaller quantities, down to 2.6 and 1.5 c.c.

For this purpose the test tubes R_1 and R_2, Fig. 3, of smaller diameter and shorter than the tubes ordinarily used, are provided, R_1 holding 2.6 c.c. of solution, R_2 1.5 c.c. The latter is fitted with a glass stopper. Two glass cylinders for submersion in the solution are further provided, of smaller diameter than the cylinders ordinarily used, to fit the narrower test tubes and adapted to be fitted in their places by screw heads. A diaphragm Mi interposed in the path of the rays adapts the latter to the reduced diameter of the

*Supplied by the firm of Schmidt & Haensch, Berlin, Prinzessinnenstr. 16, or by: American Kreuger & Toll Corporation, 114-118 Liberty Street, New York City.

cylinders. (This diaphragm is now fitted in every instrument, so that the supplementary fitting for microanalysis may be used if desired without alterations in the instrument.)

Owing to the short radius of curvature of the test tubes R_1 and R_2, Figs. 4 and 5, the light rays would on entering be so strongly deflected by refraction that the illuminated space, indicated in Fig. 4 by a circle, would become too small to yield sufficient light for observation. The test tubes are, therefore, enclosed in glass casings, which are filled with the solvent used in preparing the solution contained in the test tube. This arrangement (acting similarly to the immersion of the object glass of a microscope) diminishes the

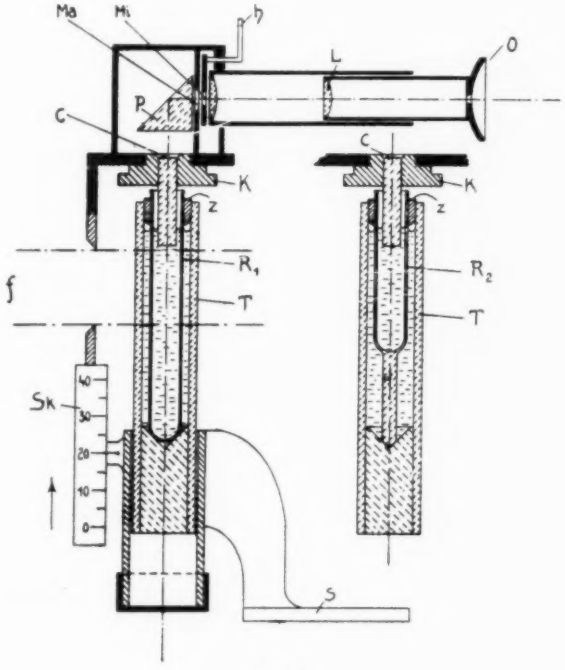

Fig. 3.

refraction, as is shown by Fig. 5, and the path of the light rays is sufficiently extended to enclose the immersed cylinder.

Test tube and casing are supplied mounted, ready for fitting into the nephelometer. The disposition of the parts will be easily understood from Fig. 3. By means of this, material which is procurable only in extremely small quantities, such as immunized sera and the like, can be subjected to investigation. The minimum quantity which can be estimated by nephelometric methods is thus reduced to one-eighth of that hitherto required.

(c) *A New Standard of Turbidity.*—In gauging turbid solutions, especially for analytical purposes, the turbid solution to be tested is usually compared with a turbid solution of known strength, the standard solution rendered turbid by the same reaction as the solution to be tested.

Suitable standard solutions for comparison are, however, in some cases difficult to procure (e.g., certain solutions of proteins). In such cases and also in chemical investigations of colloidal substances, e.g., of the variation of turbidity of the solution of a colloid during a certain period of time, it is indispensable to have a fixed and invariable standard of turbidity.

Such a standard should have the following characteristics:

1. Its strength must, when once gauged, remain constant for an unlimited period of time.

2. Its strength, or the amount of light it emits, must be capable of variation to any desired extent.

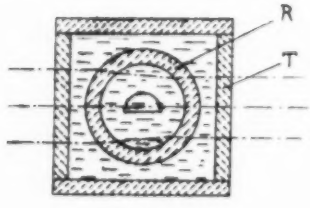

Fig. 4.

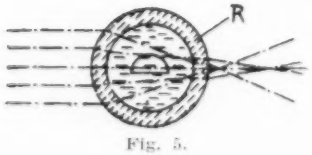

Fig. 5.

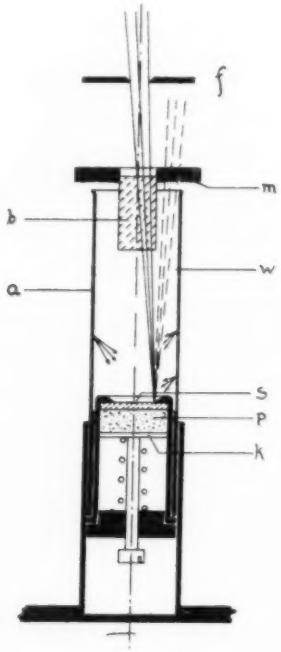

Fig. 6.

3. Its color must also be capable of variation, so that it may be made to match the shade or tint of the various turbid solutions exactly.

This latter adjustment is of importance, as the various turbid solutions, even those called white, although they are almost always of a bluish-white color, vary considerably in shade from blue to yellow. In order to be able to adjust turbid solutions accurately to equal brightness, it is absolutely necessary to have them of the same color or even shade of color, unless monochromatic light is available. There is, however, some difficulty in producing monochromatic light of sufficient brilliancy, not to speak of the inconvenience of manipulating the necessary apparatus.

A standard of turbidity consisting of a liquid or of a solution cannot fulfill the above conditions. The author therefore turned his attention to other methods of preparing a suitable and sufficiently variable standard of turbidity and finally attained his object in the following manner:

The walls of a test tube, such as is used to hold the solution in the nephelometer, are frosted in a way that allows of varying the thickness of the frosting layer or coating.

When a tube frosted in this manner is placed in the nephelometer, the frosted white surface throws diffuse light on the bottom of the tube. On the bottom of the tube a colored preparation is spread, the color of which can be varied as required. This preparation reflects colored light upwards into the immersed cylinder of the nephelometer.

The standard of turbidity prepared on this principle took the practical form shown in Fig. 6; *a* is a small glass tube which, in order to avoid light, reflected from its walls, penetrating upwards in to the submerged cylinder, is rather wider than the ordinary nephelometer tubes. The tube (*a*) is fitted on a short brass tube indicated by strong lines in the drawing. Into this brass extension of the glass tube a cartridge is introduced from below, which will be described in detail further on, and which contains the colored preparation. The whole is fitted into the base of the nephelometer tube in the same way as an ordinary tube and raised until the top of the tube encloses the glass cylinder (*b*).

The frosting of the surface of the tube is done by pouring over it a solution of collodion in ether, in which an indifferent, finely divided powder, such as talcum, is suspended. This solution, on drying, leaves a film of uniform thickness covering the surface of the glass. In this manner any desired degree of frosting can be produced by increasing or diminishing the quantity of talcum or other matter, suspended in the solution. Before the above operation is begun, the brass tube end is closed by an accurately fitting brass stopper to prevent the solution from penetrating into the interior of the tube.

When the collodion film is dry, a cartridge adapted to form the colored reflecting bottom of the standard tube is introduced into the brass tube end in place of the stopper. This reflecting bottom is formed by a powder the color of which can be varied as required and which fills the top part of the cartridge. This latter is closed off by a cover-glass (*s*), ground opaque, against which the powder is pressed by a piston (*k*). The piston (*k*) is kept in place by a spring and forms a uniform and evenly colored surface as bottom for the standard tube.

The colored powder used to form this bottom should be as fine-grained as possible. It will in most cases be of a bluish-green tint, as most of the turbid solutions are bluish-white. I have found a mixture of talcum, sulphate of copper and blue litmus very suitable, but, of course, any desired colored powder may be employed for the purpose. The color that is most suitable for the turbid medium in question must be found by experiment. The right shade can always be ascertained by a few experiments.

In case the light emitted by the colored bottom is not sufficiently intense to impart the right tone of color to the white light emitted by the wall of the glass tube, the collodion film itself may be colored or the tube may be lined with colored tissue paper. In this latter manner any desired color may be produced for the investigation of colored colloidal solutions.

In place of a glass tube which the investigator may himself frost as desired, Messrs. Schmidt & Haensch also supply a brass tube whitened inside and provided with a longitudinal slit on the side turned towards the window of the nephelometer to allow the light to fall on the whitened interior surface of the tube.

The intensity of the light emitted by the standard of turbidity may be modified, not only by varying the density of the frosting film and the brilliancy of the reflecting bottom, but also, and even more easily, in the same way as that of ordinary turbid solutions, by adjusting the nephelometer window in front of the standard tube.

The standard of turbidity should not, however, stand freely in the nephelometer as shown in the drawing, but should be raised until it touches the top fitting (*m*), in order that the bottom may always be at the same distance from the cylinder (*b*).

The immersed cylinder receives light exclusively from the bottom of the tube, that is from the cover-glass (*s*). The dimensions of the tube are such that no light entering laterally through the walls of the tube can reach the cylinder directly.

A standard of turbidity prepared in the above manner can be varied in luminous intensity and color so as to match any turbid solution and will keep indefinitely without changing.

It will probably prove useful not only in ordinary nephelometric work but also in the investigation of kinetic processes in colloidal bodies.

IV. USE OF THE NEPHELOMETER DESCRIBED ABOVE

Measurements with the new nephelometer are carried out in the following manner:

It has been proved by repeated and careful measurements that the instrument, when installed symmetrically to the source of light, is in absolute optical equilibrium, that is, both test tubes being filled with the same turbid solution and the windows adjusted to the same width of opening, both tubes show the same luminous intensity, and this state of things is not altered by exchanging the tubes. The luminous intensity of the tubes may therefore be used to adjust the source of light symmetrically in front of the instrument.

The source of light is installed as accurately as possible on a level with and symmetrically to the windows, both test tubes are filled with the same solution, both windows adjusted to the same width of opening and then instrument and lamp are carefully adjusted, so as to give uniform brilliancy over the entire field of view. The tubes a_1 and a_2 are then exchanged and, if the field of view remains unchanged, the position of the lamp and the instrument is marked on the table with chalk or a colored pencil.

Should the field of view not remain uniform in brilliancy on exchanging the test tubes, the adjustment of lamp or instrument must be repeated until the tubes can be exchanged without producing any difference.

The instrument is now ready for use:

The tubes are pressed down into the metal casing and removed out of the slides with the same. Then they are carefully cleaned outside with a chamois

cloth. The inside of the tubes should be cleaned and dried only when a series of experiments is concluded and the instrument is to be put aside for the time being. Brushes or cloths invariably leave small fibers, etc., on the surface of the glass and it is, therefore, not advisable to do anything more to the instrument while in use than to rinse the tubes well with the solution to be tested. When filled they are again fitted into the slides and raised until the solid cylinders are immersed in the solution. Great care should also be taken to keep the cylinders clean, as impurities are very apt to cause changes in colloidal solutions. Flaky precipitations were indeed often observed to form in the neighborhood of the cylinders, as a consequence of insufficient cleaning. Care must also be taken that no air-bubbles lodge under the ends of the cylinders, either in dipping them into the solution or later on, in case the temperature of the solution should rise in the course of the experiment.

The position of one of the sliding shutters being fixed and noted, the other is adjusted by means of rack and pinion, until the luminous intensity is equal over the whole field of view. The concentration of the solutions is then in inverse proportion to the openings of the windows, as read on the verniers.

The instrument indicates variations in luminous intensity with a high degree of precision. A displacement of the shutter of 0.1 mm. is distinctly observable, even with less turbid solutions.

Here, however, not only the actual performance of the instrument but also the subjective efficiency of the observer plays an important part.

The human eye which at first is insensible to fairly large differences in brilliancy, by continuous practice learns to distinguish the most delicate differences.

Thus the accuracy of the results attained in the first experiments may be greatly increased by practice.

The subjective accuracy of observation must, however, be taken into account not only generally but for every single measurement.

It is absolutely necessary to give the eye time, five to six minutes, to adapt itself to the darkness, before beginning to observe. But even afterwards, in the course of a longer series of measurements, it is advisable to let the eye rest from time to time in total darkness, as variations in its sensitiveness may vitiate the results. To avoid errors from this cause, it is advisable to carry out each adjustment several times, say ten times, which can be done rapidly and easily and to take the mean value of the observations as final result. In this way the observer can eliminate the subjective errors of observation with almost absolute certainty. On the other hand we must repeat here that it is absolutely necessary to test the adjustment of the nephelometer and the source of light before commencing operations, by filling both tubes with the same solution, as described above, even if their positions have been marked on the table previously.

The accuracy of measurement which may be attained with this instrument is very satisfactory. The average error is about 1 per cent and by practice this average may even be brought down to 0.5 per cent.

V. GENERAL DIRECTIONS FOR NEPHELOMETRIC INVESTIGATIONS

There are certain precautions that must be observed in nephelometric analysis, if successful and accurate measurements are to be obtained. These conditions which are partly analogous to those necessary in colorimetric analysis are the following:

1. The degree of turbidity of the solution to be compared must remain constant within the period of observation, nor should the solution be subject to any changes of state such as the formation of flaky precipitations. This condition is a matter of course and needs no further explanation or comment.

2. Turbid media that are to be gauged nephelometrically must be absolutely homogeneous at least to the naked eye. There is also an upper and a lower limit to the density of solutions susceptible of accurate measurement which may be easily determined empirically. If the turbidity of a solution is so slight that even a powerful beam of light produces only a faintly luminous Tyndall-cone in it, the measurements are naturally less accurate than when the Tyndall-light is of ordinary intensity. On the other hand solutions of too great density are very liable to form flaky precipitations, not to speak of the absorption of the diffracted light by the superposed liquid which in turbid solutions of high density begins to be perceptible. This shows us the necessity of studying systematically every reaction producing turbidity which we wish to use in nephelometric investigations, as to the limits of concentration which may be employed, the stability of the turbid solution, etc.

3. The difference in the turbidity or, what comes to the same thing, the concentration of solutions which are to be gauged nephelometrically should not exceed the ratio of 1:4. This is merely an empirical rule embodying the experience that with solutions more widely different in concentration measurement becomes more difficult and less accurate.

In this connection it may be noted as a most interesting fact that Lednicky[7] who, on the basis of diagrammatic sketches of the design of the above nephelometer, discussed the theory of nephelometries mathematically and was led by his calculations to the same conclusions regarding the concentrations of the solutions to be gauged.

The difference in concentration of 1:4 is, however, so great that it will never be exceeded or reached in practice. As a solution of unknown concentration is generally compared with a standard of turbidity, the difference of 1:2 between the solutions of a turbid media will hardly be exceeded in practice. Besides a greater difference can be easily corrected by diluting the stronger solution. The slighter the difference in density is between the turbid media that are to be compared, the easier will the measurement be and the more accurate the result.

4. The turbid solutions subjected to analysis must possess equal dispersive power. For, according to the formulas established by Rayleigh, the intensity of the Tyndall-light depends on two factors:

(a) The number of particles present in the turbid solution, and
(b) Their size, that is, the dispersive power of the solution.

In order to enable us to determine one of these variables the other must be constant. As we wish to measure the concentration of a solution by comparison with another, that is, the number of particles, their dispersive power must be equal.

This condition seems very difficult to fulfill; it is, however, quite possible to realize it for a number of reactions producing turbidity. In the case of inorganic substances producing turbidity, such as chloride of silver and the like, solutions of equal dispersive power can only be prepared by special devices, such as the addition of a protective colloid. With substances possessing a very large molecule such as albuminoids, alkaloids, fatty substances high up in the series, it is, however, comparatively easy to produce turbid solutions of equal dispersive power. That this condition is actually fulfilled is proved by the fact that a substance entering into reaction in the same concentration repeatedly gives solutions of equal turbidity.

This also shows the necessity of studying a reaction methodically in all respects, before nephelometric methods can be used successfully in connection with it.

5. The optical fittings of the instrument employed in measuring must be faultless in design and construction.

VI. RESULTS OBTAINED WITH THE NEW TYPE OF NEPHELOMETER. NEPHELOMETRIC METHODS

In order to ascertain the relation obtaining between the concentration of a solution and the turbidity produced, solutions rendered turbid by glycogen, chloride of silver, lecithin and other like substances, prepared by diluting an original solution in various proportions were measured by means of the above described instrument.

The positive and invariable result of all these measurements was that up to the limit ratio of concentration 1:4, measureable with this instrument, concentration and turbidity are absolutely proportional.

As a typical instance among the long series of experiments made the following may be quoted here:

Glycogen was dissolved in distilled water in such quantity as to render the solution distinctly turbid. The solution was filtered and portions diluted in the ratios marked by the whole numbers within the limit ratio 1:4. The diluted solutions were then compared with each other in irregular sequence.

The maximum error of measurement to which a beginner is liable in his first experiments is 1 per cent. With a little practice the mean error need not exceed 0.5 per cent.

The instrument itself does not show any variations in its optical equilibrium such as are experienced in the applied Duboscq colorimeter.

The manipulation of the instrument which is small and easily movable is as simple as possible.

As the reactions producing turbidity are exceedingly sensitive, they allow of determining extremely small quantities of the substances in ques-

TABLE I

SOLUTION OF GLYCOGEN DILUTED IN THE RATIOS
a:b:c:d:e = 2:4:6:8:10.

	SOLUTION B	SOLUTION C	SOLUTION D	SOLUTION E	MEAN ERROR
Average	20.06	10.02	10.12	15.0	0.3
Compared with	a = 40	a = 30	a = 40	e = 25	0.2
Ratio of dilution	1:2	1:3	1:4	3:5	+0.2
Error	0.3%	0.2%	1.2%	0.0%	0.0%
					0.42%

tion (0.0005 mg. P_2O_5, 0.05 mg. Ca, etc.). We are thus enabled to establish an entire analytical system for microchemical, especially biologic purposes.

The analytic results obtained are, provided the above-mentioned conditions are adhered to, wholly reliable and accurate and the mean error is almost always under 1 per cent. And finally the nephelometric methods are so simple and so rapidly carried out that hundreds of estimations can be made in a few hours and thus series of experiments become possible that would be totally impracticable, if they were to be carried out by the ordinary analytic methods. As an example of nephelometric analysis, the estimation of phosphoric acid[1] may be quoted, which has been generally adopted in biologic work and has been used, e.g., by Willstaetter in his work on ferments for the estimation of extremely small quantities of phosphoric acid. The analytic method allowing the estimation of extremely small quantities of calcium, described by P. Rona and H. Kleinmann[12] has also proved useful in a biologic work. The same authors have further elaborated a method for the analysis of proteins in extremely small quantities[13] which has proved useful in following the process of fermentation. By means of this method the action of pepsins on serum-albumin could be successfully studied; an investigation, which had hitherto offered great difficulties, owing to the want of a suitable analytic method.[14]

P. Rona and H. Kleinmann[15] have developed a nephelometric method for estimating casein which is used in the investigation of peptic and tryptic processes by fermentation. This method is based on the turbidity produced by quinidine in solutions of casein. The investigation of the phenomena and processes produced by diastatic ferment have also been facilitated by the employment of nephelometric methods. P. Rona and Van Eweyk[16] have described a nephelometric method for the investigation of the action of amylase on glycogen.

Thus nephelometry though still in the initial stage of its development has already proved eminently useful in microchemic and biologic research. It is, however, all the more important to be careful not to get an intrinsically valuable analytic method into disrepute by employing it injudiciously and indiscriminately. It is especially in physiologic chemistry, where substances have often to be subjected to analysis in extremely small quantities, that nephelometric methods promise to be of the greatest importance and advantage. On the other hand, nephelometric methods may, owing to the high precision of the instrument, be used with advantage in purely colloidal work. For from

Rayleigh's formula it follows that both the volume and the number of the smallest particles of a colloid in solution are proportional to the intensity of the light they emit and, the strength of the solution being known, may therefore be subjected to measurement.

Generally speaking changes in the colloidal state may be detected and gauged by variations in the dispersive power and consequently in the intensity of the light emitted.

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DIFFUSE CORTICAL SCLEROSIS*

A Clinical and Pathologic Report of Two Cases

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San Francisco, Calif.

IN MARKED contrast to those types of cortical sclerosis plainly secondary in character and associated with vascular changes or inflammatory reactions, or to tuberous sclerosis or to the patchy multiple sclerosis there is occasionally found a diffuse cortical atrophy and sclerosis of quite unknown etiology in which there is a uniform atrophy with destruction of the cortical nerve cells and marked overgrowth of the glial tissue. This type of sclerosis is commonly found in children and appears to represent a disease entity both clinically and anatomically.

We have had an opportunity to examine two such cases. For the clinical data and material of the first case we are indebted to Dr. L. Emmett Holt and to the former resident physician of the Babies' Hospital, New York, Dr. Dorothy M. Reed; the second case is from the Children's Hospital of San Francisco and the clinical record was kindly given to us by Dr. Edith Bronson.

Under the title of "diffuse sclerosis of the brain" one finds included almost every condition showing a glia proliferation,—cases of dementia paralytica, sclerosis due to arteriosclerosis, hydrocephalus with secondary atrophy and sclerosis, tuberous sclerosis, multiple sclerosis, amaurotic family idiocy and various conditions appearing in the literature as meningoencephalitis. A generalized atrophy of the cerebral cortex in children whose history gives no lead as to etiology is evidently a rare condition; Ziehen[1] makes the statement that outside of the motor cortex and occasionally of the speech and sensory areas he cannot report any cases of general cortical dysplasia.

Oppenheim[2] mentions that diffuse sclerosis occurs in various conditions and with different pathogenesis and nosologic significance. Heubner[3] apparently gave the first description of diffuse cerebral sclerosis in children; the child died at the age of five years; in this case there is a question of the etiologic relationship as the child was normal, except for deficient speech development, until he had a head injury at the age of three and three-fourths years; on section it was noted that the brain cut like fresh Swiss cheese. The author looks upon the condition as not congenital but developing in a previously healthy individual.

Schmaus[4] reports the findings in a three-year-old child who had been well until one and three-fourths years old; the brain, especially the convolutions, were markedly atrophied. In this case, however, the child had muscular cramps and fever at the onset and a pachymeningitis interna was

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Received for publication Nov. 29, 1926.

found; the pathologic changes were therefore evidently the result of some inflammatory process.

In the condition described by Strümpell[5] there was an overgrowth and shrinking of the neuroglia; the ganglia cells were apparently normal; the cerebrum was one-third of the normal size and the cerebellum of nearly normal size. Since a general perivascular infiltration was found the process was possibly a chronic encephalitis.

Under the title of "Diffuse Cortical Sclerosis of the Brain in Children" Bullard[6] reports a case and discusses the relationship of this condition to head injuries. His patient was a boy aged thirteen years. He regarded the hydrocephalus as secondary to a diffuse sclerosis. He says "Schmaus' case is somewhat analogous," but thinks "it should be classed among the lobar scleroses which may be very diffuse."

Marchand and Nouët[7] state that the pathologic picture they term "la sclerose cerebrale superficielle diffuse" may result from a chronic meningitis due to various intoxications or infections; it may be an anomaly in development of the brain, a defect in equilibrium existing from birth between the nerve cell elements and the neuroglia tissue. It may develop in a previously normal brain under the influence of a toxin or infection without concomitant alteration of the meninges or cortical vessels. He noted an alteration of the tangential fibers.

Krabbe[8] describes a "new familial infantile form of diffuse brain sclerosis" in which the changes are almost entirely in the white matter. There is a replacement of the destroyed tissue by neuroglia with relative intactness of the nerve cells.

Two cases of diffuse cerebral sclerosis were reported by Moser,[9] one a nine-year-old boy and the second a twelve-year-old girl. In these cases the histologic examination indicates an inflammatory alteration in the sense of Schmaus.

REPORT OF CASES

CASE 1.—The patient was a female child aged two years and ten months at the time of its admission to the Babies' Hospital. Its hereditary and familial history is quite negative and unsuggestive; birth was at full term with normal delivery. There was no evidence of lues; the child had developed normally up to its present illness; it was nursed for four months, then fed on diluted cow's milk, and since one year of age had taken table food. The child cut its teeth beginning at one year, walked well at eighteen months, and talked words. There is no history of contagious diseases or gastrointestinal disorder.

The present illness began insiduously when the child was somewhat less than two years of age. At first it was noticed to stumble and fall; the condition gradually increasing so that after about four months it could not walk or stand; it could, however, still creep. For six months previous to admission the child had been helpless, not being able to use its feet at all and later becoming uncertain in the use of its hands. Speech and understanding went in the same gradual manner until the child became "idiotic" as well as helpless and showed impairment of hearing and sight. For six months previous to the hospital entrance there had been constant movements of the hands and feet. No definite convulsions were noted but the child would jerk suddenly and stiffen out. There was no fever, vomiting or headache; while under observation there was no attempt at sphincter control.

Physical examination by Dr. Holt; "Stout, well nourished child, good color, no wasting. Almost constant movements of hands, arms and fingers of a general athetoid character, somewhat resembling choreiform movements. Lower extremities are not paralyzed and movement of legs are somewhat similar to those of arms. At times a well marked tremor of feet and toes. Slight ankle clonus. Knee jerks very much increased and there is rigidity more than relaxation. No actual or apparent atrophy. Eyes: Pupils normal, react to light. There is slight obliquity of the head and flattening over left temple. Skull otherwise negative. Slight internal strabismus. Normal teeth and gums; clean tongue."

"Mentally: Like child in active delirium. Marked symptoms of cerebral irritation; grinding teeth, rolling head; no rigidity of neck; sees and follows objects. Hearing is doubtful. Symptoms everywhere bilateral. No separation of sutures of skull. Pulse varies from 120-80, when she is restless or quiet. Lies quietly asleep for a few minutes then starts suddenly as if electrified."

The fundi were examined on two occasions by Dr. Marple who reported incomplete atrophy of both discs. No cherry red spot observed.

Antisyphilitic treatment was begun but without results. Lumbar puncture yielded a clear culturally negative fluid. No cytologic examination was made.

The neurologic progress of the case (slightly less than two months till death) was marked by periods of apathy alternating with states of wild excitement, with incoordinate bizarre movements involving all the extremities, especially the arms and to a less extent the trunk. The excited periods were at times precipitated by disturbing the child and were more marked during the infections from which the child suffered towards the end. Later the movements took on a rhythmic character, varying from four to seventy per minute. About a week before death the child lay on its back, arms extended at the sides, thighs abducted and knees flexed on thorax, legs flexed on thighs; very little rigidity; no Kernig's sign. Knee jerks not obtained; quite marked foot drop. No ankle clonus; no Babinski's sign. No opisthotonos. Tache cerebrale well marked.

A series of infections supervened. A tonsillitis, later a double purulent otitis media which ruptured, and finally terminal bronchopneumonia. During the febrile periods, erythematous rashes appeared; they were more or less transitory and occurred on the chest and abdomen. Periods of pallor or cyanosis, likewise transitory, occurred. Respiration towards the end became irregular but not of Cheyne-Stokes' type. Feeding was by gavage and was well retained. Irregular vasomotor disturbances about face and neck were noted late in the disease and the rash over the chest and abdomen towards the end assumed a macular, punctiform character lasting twenty-four to forty-eight hours, and then completely disappearing. Death with bronchopneumonia and general inanition.

The diagnosis of the neurologic condition was in doubt. Dr. Pierce Bailey suggested amaurotic family idiocy, and one of us (G. Y. R.) who saw the case on one occasion through the courtesy of Dr. Reed, was impressed with the marked ataxic character of the movement and taken together with the optic atrophy, suggested a tumor involving the cerebellar apparatus. Neither, however, was substantiated by the anatomic findings.

The necropsy was performed by Dr. Martha Wolstein. The brain together with a portion of the cervical cord was fixed in formalin.

The fixed brain presented a symmetrically and diffusely thick, grayish, boggy, pia-arachnoid. This membrane is so thick that no adequate idea of the fissuration is obtainable while it is in place. In removing the pia it is found to be tough and the stripping shows a distinct tendency to tear the surface of the cortex, not causing small punctate defects, but distinct and extensive defect of tissue. Roughly one-third to one-half the cortex appears to come away. The cortex superficially is distinctly soft and spongy throughout, with little variation over the whole surface, except that the condition was especially noticeable over the central convolutions and in the calcarine areas. The pia being partly stripped, the underlying cortex showed a high grade of diffuse symmetrical atrophy, slightly more accentuated frontally. The sulci are wide, yet shallow, and the convolutions shriveled, soft to the touch superficially, but on deep palpation there is an increased elastic firmness to the tissue. (Figs. 1 and 2). This is particularly brought out on sectioning, the tissue cutting as if

rubber were incorporated with it. There is no abnormality of convolutional distribution. No patches of discoloration or evidence of focal lesion of any type were encountered. The cerebellum was symmetrical, the folia somewhat shrunken, the pia not nearly so gray and thickened as that of the cerebrum, though here also somewhat adherent. The vessels at the base were normal in appearance and distribution. The floor of the fourth ventricle showed no granulations. The cranial nerves appeared normal throughout, except the optic nerve which seemed somewhat small but did not show the grayness of atrophy of high degree.

The microscopic examination of the material brought out a remarkable diffuse neuroglial hyperplasia occurring throughout the cortex and to a much less extent in the white matter of the brain and central nuclei; secondly, a peculiar alteration of the nerve cells,

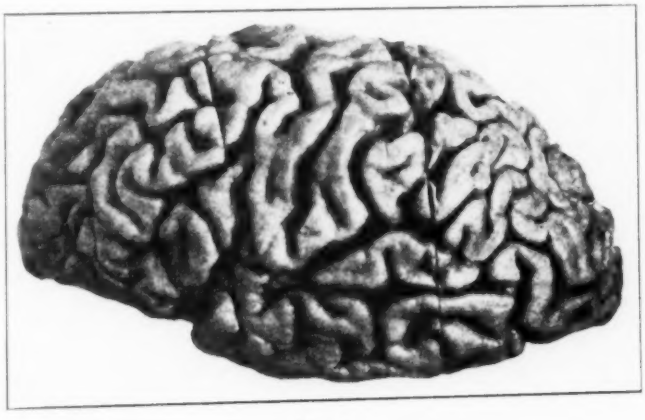

Fig. 1.

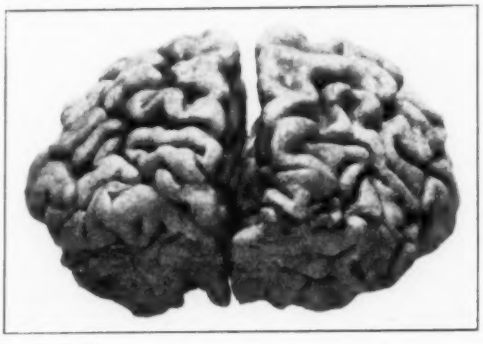

Fig. 2.

Figs. 1 and 2.—Photographs of brain of Case 1.

not only of the cortex but involving the nerve cells generally throughout the central nervous system; in the third place an absence of tract degeneration except some thinning of both crossed pyramidal tracts; fourthly, an absence of inflammatory elements in the histologic picture, and lastly, an hypertrophy of the pia, probably entirely compensatory in nature.

The principal or at least the most striking feature of the microscopic picture is the glial hyperplasia diffusely affecting the whole cortex; no part escapes; even where the cortex is rolled up to form the fascia dentata, glial hypertrophy occurs. In fact the process is so diffuse and evenly distributed that it would be more than superfluous to describe the various small sections in detail. Sections stained for neuroglia by Mallory's phosphotungstic hematoxylin, show the superficial layer of glia as a more or less thickened felt work, from which here and there bunch-like masses of fibers entwine and mingle with the connective tissue of

the pia. In the molecular layer the glial cells are increased in size and are rich in fiber formation, but beneath this zone, scattered diffusely, are large protoplasmic rich, often bi- or tri-nucleated neurolgia cells with a remarkable wealth of processes which are margined by, and through the centers of which run, numerous fibrillae giving the characteristic reaction of neuroglial fibrils. (Figs. 3 and 4.) The processes may frequently be traced to vessels about which they twine forming a loose mesh-like appearance which probably is largely an artefact and which corresponds to the line of tearing noted in the gross description.

Fig. 3.—Neuroglia—phosphotungstic acid hematoxylin. (Case 1)

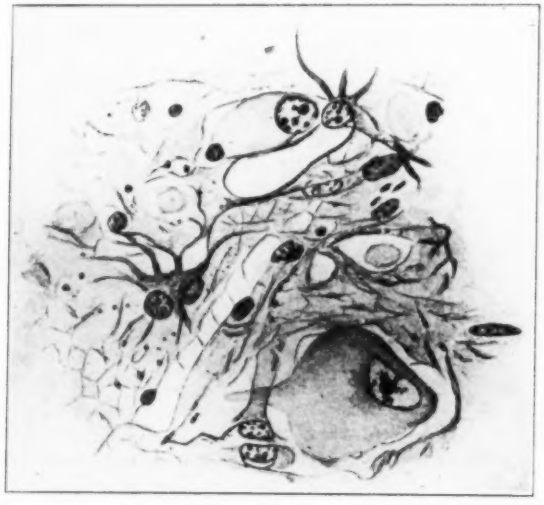

Fig. 4.—Neuroglia—phosphotungstic acid hematoxylin. (Case 1)

Considering the anchoring of the pia to the cortex by the superficial glia and similarity of the cortex to a layer of raw cotton in which nerve cells lie embedded, an artefact of this type may be explained. It is especially noticeable that very few of the small naked satellite glial cells are found, all having undergone the hypertrophy. With all the glial hypertrophy no mitotic figures were encountered.

In the immediate subcortex again the glia is hypertrophic though less conspicuously so. It is unfortunate that Mallory's phosphotungstic acid hematoxylin stains the myelin, making search in such regions quite difficult, but in the modified Nissl stain evidence of

similar hypertrophy is distinct. The degree gradually diminished but it is present generally in the basal ganglia and thalamus. In these situations the hypertrophic glia often appear as long, thin cells with tapering ends either curved or straight, angular or branched; the nucleus is centrally situated, long and narrow with rounded ends, and contains a few chromatin particles. These correspond to the rod cells of Nissl which are of not unusual occurrance in general paralysis of the insane, and less frequently in senile dementia. In this region many small, deeply-stained glial nuclei of the usual type are seen in contrast to the cortical findings.

The glia of the cerebellar cortex is hypertrophied, Bergman fibers being irregularly seen as thick branches running through the molecular layer. The general picture, however, does not approach the severity of the condition found in the cerebral cortex.

The neuroglia of the spinal cord does not show any definite hypertrophy; even in the region of the crossed pyramidal tracts, which as we shall see are diffusely thinned, the reaction fails.

Fig. 5.—Cortical ganglion cell. (Case 1)

On the part of the nerve cells marked changes are found. The abnormality is of one type but varies in degree depending to a considerable extent on the size of the cell. The nervous elements of the whole cerebrospinal axis are affected and a morphologically normal cell is rarely seen.

One may best obtain an idea of the abnormal changes in the cells by observing one of the larger cell types, an anterior horn cell of the spinal cord or a large motor cell from the paracentral region. Primarily the change may affect the portion of the cell from which the axis cylinder arises, and consists of a breaking up of the Nissl bodies, giving a diffuse dusty appearance to this portion of the cell; the change is accompanied by a local swelling of the cytoplasm (Fig. 5).

Few cells show so early a change, but most have proceeded to a stage where the Nissl bodies have disappeared from the swollen portion of the cell leaving a finely reticulated or, perhaps, vacuolated appearance locally, in which there is a yellowish cast to the cytoplasm, strongly suggesting a prepigment stage. The swollen portion of the cell varies in amount within wide limits. In the remainder of the cell the Nissl bodies are well preserved, and extend normally along the dendritic processes.

The nucleus in the larger cells appears hyperchromatic and as a rule is situated in that portion of the cell which contains the Nissl bodies; it is often surrounded by an especially marked layer of Nissl substance which may obscure the nuclear membrane. As the size of the cell diminishes the relative size of the area of swollen, Nissl body-free cytoplasm increases; this statement holds particularly true for the cortex where in the small pyramidal cells we have an extreme example of the end stages of the process. There, what were formerly small pyramidal cells are swollen, bulbous or nearly circular bodies with all trace of chromatic substance wanting, the cytoplasm of a yellowish tinge and reticulated. The nucleus undergoes a progressive diminution in size, appearing not as a circular body but as a little irregular dot, sharply circumscribed body with no internal differentiation, lying usually against the cell wall. As described above the size of the cell rather than the position seems to predicate the degree of the reaction, but also there seems to be some relation to the degree of glial hypertrophy and the stage of change reached by the nervous elements, most marked in the cortex and less so in the basal ganglia where the cells do not reach the more extreme grade of reaction. The central nucleus of the thalamus for example, shows very uniformly its cells shaped like acorns, with bulbous bases of reticulated cyto-

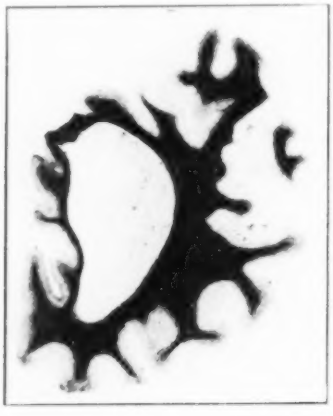

Fig. 6.—Section of cerebral hemisphere stained by Weigert's myelin sheath method. (Case 1)

plasm; and the nuclei diffusely hyperchromatic, compressed into the beginning of the apical processes and closely surrounded by a small quantity of dense chromatic material. Equivalent results would follow description of cells from other regions, the cerebellum, the medulla, or any of the many cortical regions observed. It might be stated that as a group probably the cells of the posterior olfactory regions and the dentate fascia show less change than those of any other functional system.

We now come to the picture presented in the tissues stained by Weigert's myelin sheath method. (Fig. 6.) Surprisingly little abnormal is revealed by it. In the cortex the tangential fibers are generally present though separated more than normally, apparently by the great glial hypertrophy. The radial zone is less myelin rich than the normal adult cortex, but in the white substance, no degenerative change is of sufficient extent to make itself visible. Especially to be noted is the intactness of the optic nerves and tract, showing that what there was of visual dimness must either have been due to retinal or cortical disturbances. This cortex shares the fate of all the remainder and to a marked degree; the retinae were unfortunately not available for examination. In the spinal cord, however, there is a diffuse though slight thinning of the crossed pyramidal tracts. This may represent a progressive retrograde degeneration of some of the long fibers beginning distally from their cells.

As to the mesodermal elements little can be said. The thick boggy pia shows simply an increase of the connective tissue elements, and nothing of a chronic inflammatory character. There is, however, a rather striking pigmentation of the leptomeninges consisting of a golden yellow pigment contained in phagocytes as well as similar pigment apparently lying free. The vessels also are negative, both in the pia and in the cortex. There is no hypervascularity. The perivascular spaces are prominent and the relation of the hypertrophied glia to them has already been noted.

CASE 2.—Clinical History by Dr. E. Bronson. E. C., male, aged ten months, was admitted to the Children's Hospital, March 7, 1921, with the complaint of vomiting and making no effort to sit up.

Family History: Neither father nor mother is strong. A sister, five years old, is healthy. No family history of tuberculosis, syphilis or insanity.

Past History: Full term normal birth, though prolonged. Weight six and three-fourths pounds. Breast fed without difficulty for two to three months but taken from breast then because of failure to gain. Later feeding was on goat's and cow's milk formulas. The baby was never hungry and took only about one-third of the food offered. Vomiting, not projectile, started soon after the baby was taken from the breast. Milk of magnesia was given daily for constipation.

Mother had noticed that he did not know her from a stranger, that he did not pay attention when she was preparing his bottle, that he cried when she held him and preferred to be left alone. He never smiled, or turned his head toward a light or seemed to notice a sound.

Examination: Weight fourteen pounds, six ounces. A much undersized but not greatly undernourished infant. Color good.

Head, Suture lines palpable. Fontanelles were closed. Hair abundant and long. Head measurements showing general reduction are given below.

Eyes, Pupils react to light but the eyes will not follow a light. Winks when a bright light is suddenly flashed in the face. No strabismus or nystagmus. Eye grounds are negative; no cherry red spot.

Ears, negative. Teeth, two lower incisors. Tonsils small, lymphoid glands not enlarged. Lungs, a generalized bronchitis. Heart, negative. Abdomen, negative. Genitalia, testicles undescended.

Urine, negative.

Blood, Reds 4,900,000; whites 9,800. Differential, polymorphonuclear 65, lymphocytes 32, large mononuclear. 3. Von Pirquet negative.

Cerebrospinal fluid, 10 c.c. of blood-tinged fluid not under pressure, Wassermann negative.

Special Condition: An exceedingly spastic infant, showing at times partial relaxation. Legs were not crossed but extended stiff with the toes pointed, and arms were held flexed at elbow. At times he went into moderate opisthotonos position, again the neck was quite relaxed. The deep reflexes are all much exaggerated. On any attempt to elicit the Babinski or ankle clonus he went into general clonic movements, arms and legs rapidly flexed and extended, and very striking clonic movements of the chin. A cephalic cry accompanied these movements; when quiet the child always showed spastic extremities, yet neck and trunk muscles were often relaxed.

Yawning was characteristic and frequent, a complete yawn, mouth opened, legs extended, elbows flexed. No athetoid movements were noted and there was no ataxia. He screamed frequently, always if touched. It was impossible to test sensation, as handling brought on a general reflex motor reaction.

He was in the Children's Hospital for three weeks. His bronchitis improved. The bowels were loose rather than constipated. He vomited very little. Feeding took much

patience and he gained only five ounces. He had vegetables, cereal, etc., as well as milk, when taken home.

Diagnosis: Microcephalus, congenital cerebral aplasia.

When sixteen months old, in August, 1921, he was admitted to the Pediatric Service of the University of California Hospital because of failure to gain. The head measurements were practically unchanged. He had no more teeth. The pupils were equal and reacted to light. The neck had become constantly rigid, and abdominal muscles tense. The

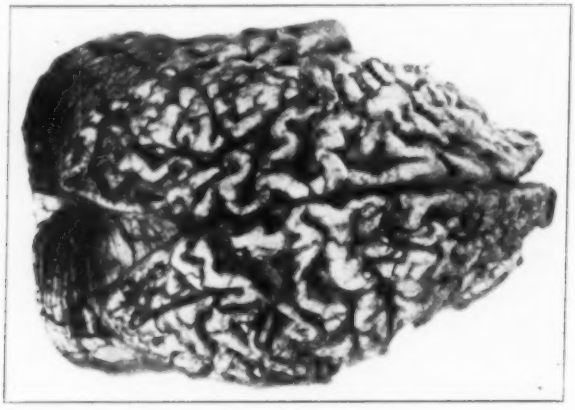

Fig. 7.

Fig. 8.

extremities were very spastic except the right leg which was flaccid and atrophic with absent tendon reflexes. According to the nurse's notes, he screamed a great part of the time.

Radiogram of the skull showed general thinning with no distinct suture lines visible.

Blood, hemoglobin 51 per cent and red blood count 2,720,000.

Wassermann, negative, both antigens.

Von Pirquet, negative.

Diagnosis microcephalus.

Head measurements were generally diminished as compared to the average normal.

He was in the hospital four days only.

He was brought into Dr. Bronson's office on Oct. 21, 1921, at the age of eighteen months because of wasting and sent again to the Children's Hospital. His weight was two

pounds less than in the preceding March. The examination of the nervous system showed even more striking spasticity than before, the muscles of neck and back standing out like cords, with the exception of the right lower extremity. The right leg, thigh, and buttock muscles were atrophied and the leg shorter than the left. The reflex clonus noted on the first admission was no longer present. The clinical picture was that of spastic diplegia, with lower motor neuron paralysis of the right lower extremity, only. The deep tendon reflexes

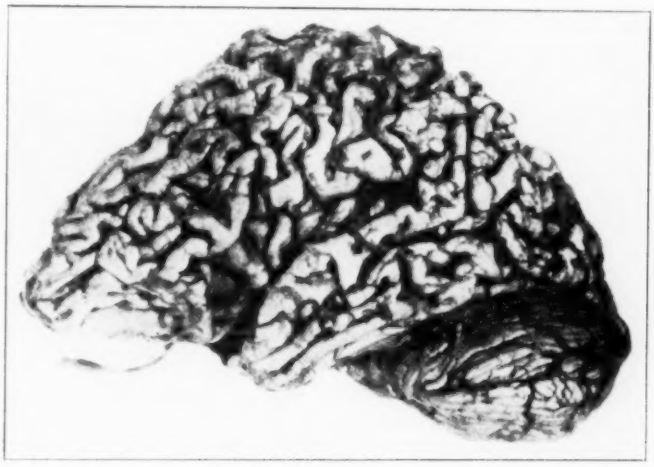

Fig. 9.

Fig. 10.

Figs. 7, 8, 9, 10.—Photographs of brain of Case 2.

were absent on the right. No history of the time of onset of the flaccidity could be obtained. He died apparently of diarrhea.

Clinical observation of this child recalled Sherrington's description of the decerebrated cat. The behavior was distinctly that of the reflex animal. The etiology could not be determined. A pachymeningitis with proliferation of the meningovascular tissue, and secondary cerebral involvement, or a primary aplasia of the cerebral tissue with secondary meningovascular proliferation were possibilities. Tay-Sachs' disease was ruled out by examination of eye grounds.

In regard to the lower motor neuron paralysis, acute poliomyelitis, localized hemorrhage, or a focus of infection secondary to the upper respiratory infections, was considered.

The autopsy was performed by Dr. Rusk and the anatomic diagnosis was as follows: Microcephaly. Diffuse atrophy of the cerebrum and the cord. External hydrocephalus. Pulmonary tuberculosis. Shortening and atrophy of the right leg. Undescended testicles.

In the detailed report of the nervous system it was noted that "the dura is very firmly adherent to calvarium and when torn away the bone shows reddish areas in the diploe. The dura is not thickened but on either side of falx there is a translucent gelatinous pseudomembrane about 3 mm. thick most marked on the right side where it shows laminations. There is marked external hydrocephalus filling a space about 3 cm. between the dura and the atrophied cerebrum. There are about 250 c.c. of fluid which is normal in appearance.

The brain weighs 160 gm; the length of the right hemisphere is 10 cm. and of the left hemisphere 9.5 cm.; the width at the base of the brain is 7.4 cm.; the total width of the cerebellum is 7 cm. The gross appearance of the cortex of the brain resembled the surface of a pecan nut; the gyri were narrow and the sulci were wide; it is apparent that the convolutions developed normally and no marked pathologic changes occurred until after the convolutions were well formed. (Figs. 7, 8, 9, 10.)

Base of the Brain: The vertebral and basilar arteries show no abnormality. The circle of Willis is negative except that the internal carotid arteries are smaller than normal. The middle cerebral branches are easily seen as they pass through the gaping sulci of the fissure of Sylvius. The arterioles are not injected and the veins are not distended. The hippocampal gyri and uncl stand out very prominently. The gyri orbitales and recti of the frontal lobes show extreme atrophy and are narrowed to 1 cm. in front. The cerebellum is much larger proportionally than the hemispheres and seems well formed, but the left lobe is larger and slightly darker in color than the right. The pons, medulla, basal ganglia and cranial nerves are not remarkable. The optic nerves are small. The pia is negative.

Superior and Lateral Surfaces: The right hemisphere is 0.5 cm. longer than the left. The longitudinal fissure is wide and at the tip of the occipital lobes measures 2.5 cm.; at the tips of the frontal lobe it measures 1 cm. The vessels and pia are negative. The convolutions are perfectly formed but there is a uniform atrophy with widening of the sulci and the entire cerebrum feels like tissue hardened in formalin. The central fissure is very prominent and the fissure of Sylvius measures at the surface from 0.5 cm. to 1.5 cm. in width.

The cord is small, firm and shows a depression just anterior to the posterior horn in the right. In the lower thoracic and upper lumbar regions there is a hemorrhagic exudate beneath the dura, especially about the nerve roots with slight adhesions between the pia and dura.

MICROSCOPIC EXAMINATION

Cerebral Cortex: An intense and fairly uniform proliferation of the glial elements is found throughout the cerebral cortex so marked that nerve cells are found with difficulty. Many types of gliae cells are present varying from the small cells with a faint margin of cytoplasm around the nucleus to enormous protoplasmic glia cells. A number of elongated gliae cells are to be seen, some of them definitely rod shaped. Neurogliar stains show a striking network of glia fibrilla; this network is rather close and in some areas very dense, though sometimes forming sieve-like areas. Small glia nuclei are fairly numerous in this network of fibrils.

In many areas the processes of the gila cells can be traced to the blood vessels, at times the intertwining of the fibrils forms a zone of denser sclerosis around the vessel. Along the margin of the cortex and on the ventricular surface glial fibrils form a closer reticulum than in the deeper parts of the cortex; this zone of marginal sclerosis is narrow in most sections of the cerebrum. This profuse overgrowth of neuroglia along the border forms in places a tuft which extends toward and into the pia.

Scattered throughout the cerebral cortex are clumps of cells about the size of small glia nuclei; (Fig. 11); there will be 15 to 30 or more of these cells in a group and they are most commonly situated near the margin of the cortex. In some of these cell clusters there is a meshwork of fibrils that resembles an Alzheimer plaque, as occurs in Alzheimer's disease.

In some sections of the cortex, especially in the occipital region, there are wide meshes in the glial network forming a line of lacunae at the junction of the gray and white matter.

There is a striking paucity of nerve cells throughout the cortex. In many sections no nerve cells are to be found and no normal nerve cells are to be seen in sections from the

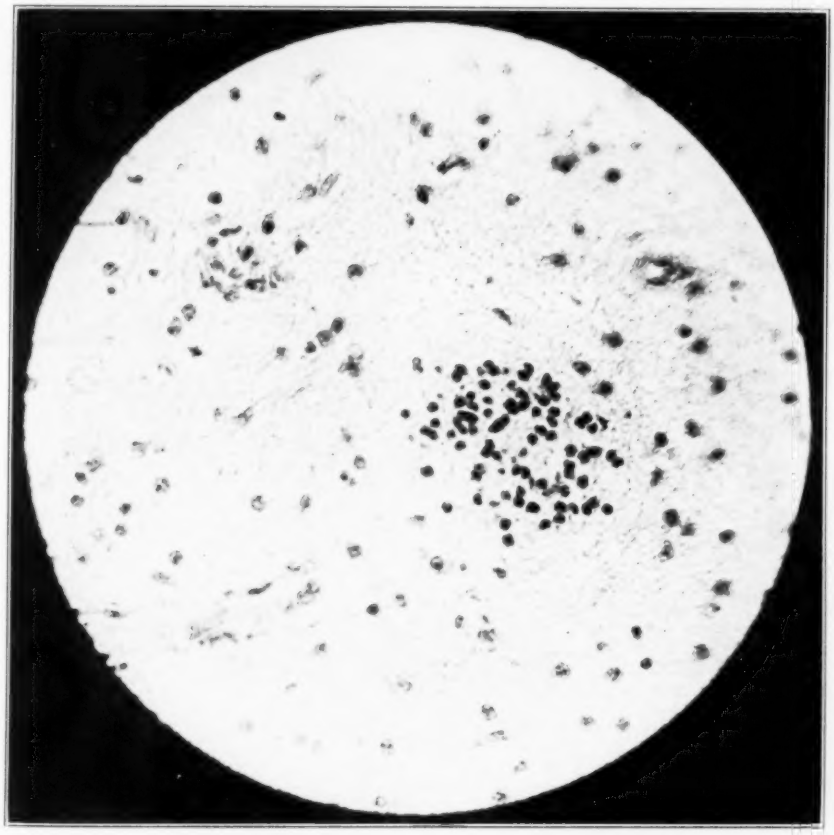

Fig. 11.—A clump of glia cells. (Case 2)

cerebral cortex. In the most nearly normal cells the nuclei are enlarged and somewhat eccentrically placed; the nuclear substance is arranged around the periphery of the nucleus; the chromatin substance of the cell is rather diffusely stained and there is seldom seen the well defined Nissl bodies present in normal nerve cells. (Fig. 12.) The axonal portion of the ganglion cells is very faintly stained and in cells showing more advanced chromatolysis no processes at all are to be seen.

In cells where the pathology is only moderately advanced the nucleus is always swollen and shows the peripheral arrangement of the nuclear material; as a rule the more marked the chromatolytic changes the more eccentrically placed is the nucleus. As the disintegration of the cell progresses it becomes more circular in outline and usually stains rather faintly and diffusely, presenting a poorly defined reticulated cytoplasm; occasionally there is well

marked axonal type of cell alteration with the chromatin substance placed at the periphery of the cell.

Although the so-called neuronophagia is usually noted with the more advanced grades of chromatolysis and satellitosis is not infrequently seen this process is not as marked as often found in other types of pathology showing a marked destruction of the nerve elements.

In the white matter of the cerebrum there is marked proliferation of the glial nuclei and fibers; running parallel with the myelin sheaths there are rows of glia cells apparently

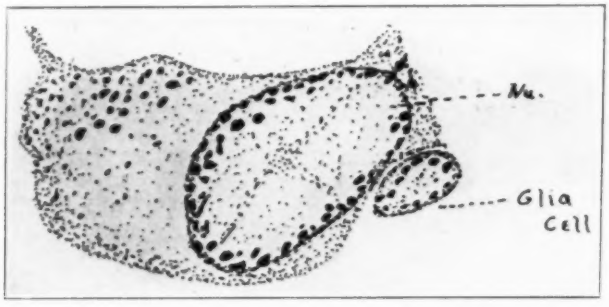

Fig. 12.—Most nearly normal nerve cell found in cortex of brain of Case 2.

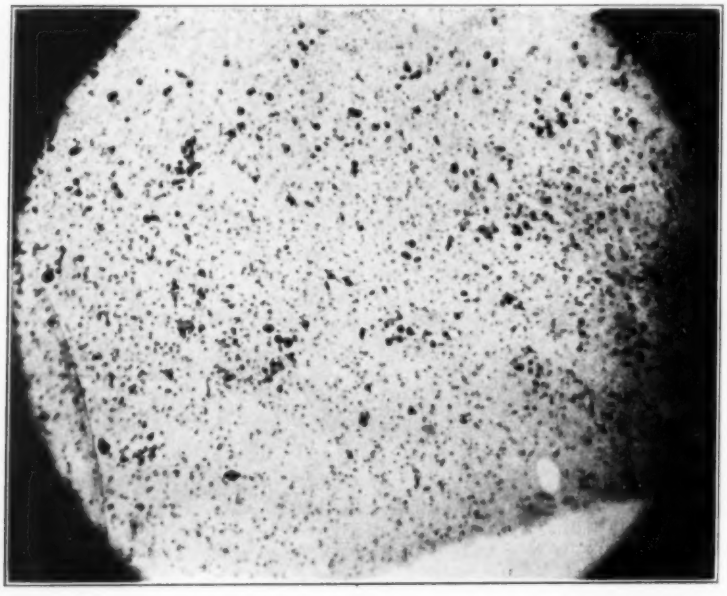

Fig. 13.—Section of cortex of Case 2 stained for fat (scharlach r stain), showing numerous fat granule cells.

in the space of destroyed nerve fibers. In some areas a number of large vacuolated glia cells are present and in sections stained for fat there are many "Gitterzellen" seen filled with fat globules. (Figs. 13 and 14.) The glia cells in the white matter, as a rule, have larger nuclei and more cytoplasm than those of the cortex. In cross sections one or more glia cells are seen within a faintly stained myelin sheath.

The myelin sheaths are greatly diminished towards the cortex; in sections stained for medullary sheaths almost none is seen grossly in the gyri and there are only a few places in the corona radiata where there are sufficient myelin sheaths to be shown grossly.

Microscopically there is occasionally seen a myelin sheath in the gyri and increasingly greater numbers in the deeper parts of the cerebrum (Fig. 15); the medullary sheaths are very irregular in outline and appear in various stages of disintegration. The myelin sheaths of the internal capsule are generally faintly stained and irregular in outline and they vary somewhat in size.

In the basal ganglia there is an increase of the glia cells. Clumps of cells and fibrils similar to those described in the cortex are found in the ventricular nucleus. There is a marginal sclerosis along the ependyma and also a widening of the perivascular spaces. Occasionally there is seen a vessel with an enormous space partly filled with a glial network. The nerve cells of the basal ganglia show chromatolytic changes but much less than those in the cortex; a common picture is a swollen nucleus with an irregular distribution of the nuclear substance surrounded by a granular chromatic material and at various distances beyond this the faint outline of the cell.

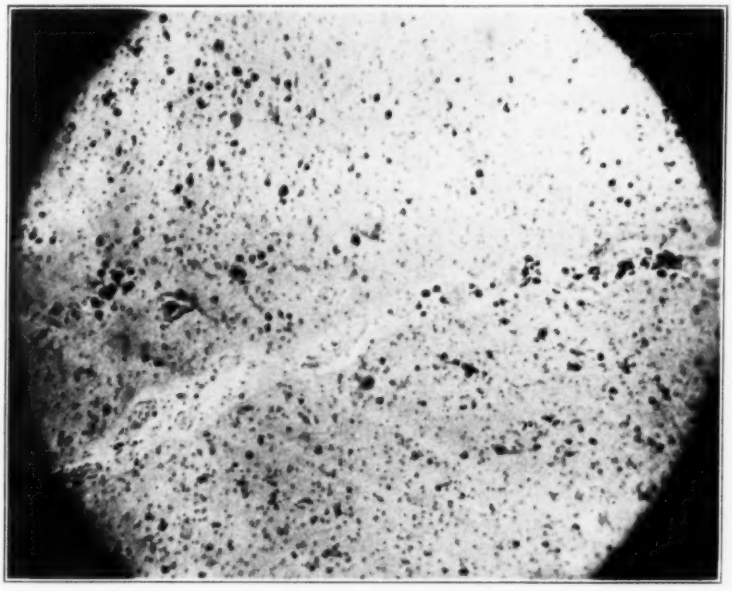

Fig. 14.—Section of cortex of Case 2 showing accumulation of fat granule cells along blood vessel. (Scharlach r stain, counterstained with hematoxylin.)

At places along the ependyma there is a peculiar break of the ependymal cells; these areas have the appearance of being pushed out and in some of them there is a definite increase of the glial fibrils.

In the midbrain marginal sclerosis is intense in some areas, especially in the region of the pyramidal tracts. The extension of the glial fibers into the meninges is striking in these sections. There are immense tissue spaces around some of the vessels and perivascular gliosis is frequently seen. The nerve cells of the third and fourth nuclei and the mesencephalic root of the fifth show varying grades of chromatolysis to the most extreme but there are many more nearly normal cells than in the cerebral cortex and some cells that appear normal. In the involved cells the changes are of the same type as described in those of the cortex.

The gliosis in the pons and medulla is not as marked as in the cerebrum but in some areas there is a fairly dense marginal sclerosis and just central to this there is often noted a loose glia network. Moderate chromatolytic changes are seen in some of the cells in the olive but many of the cells of this structure appear quite normal.

The cerebellar cortex shows some increase in glial fibers but no striking pathology is seen. In the cervical region of the spinal cord there is a netlike glial formation at the margin, especially in the regions of the dorsal cerebellar tracts. Definite, though not marked, changes are present in the anterior horn cells; the nucleus is more involved than the cell processes. The cell cytoplasm is diffusely stained but with clumps of tigroid substance scattered through it; in many of the cells a definite nuclear outline is lacking. Occasionally a cell is seen with the processes fairly distinct but with the cell body nearly disintegrated, and especially that part of the cell giving off the processes. These cells may indicate the course

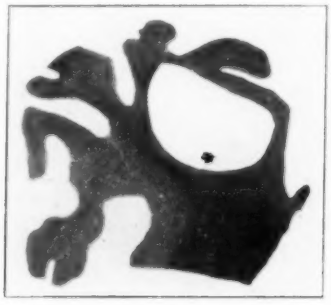

Fig. 15.—Case 2—Myelin sheath stain of cerebrum showing absence of sheaths in gyri.

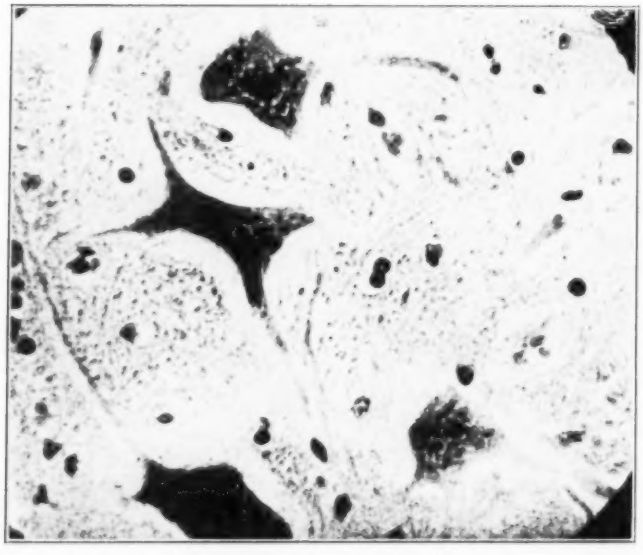

Fig. 16.—Anterior horn cells. (Case 2)

of the pathologic process, the cell being primarily involved. There is a moderate satellitosis around the anterior horn cells. (Fig. 16.)

In the anterior horn on one side of the lumbar cord there is a striking abnormality; it is about the size of the opposite anterior horn and is well demarcated from the denser white matter of the cord. With the glia stain it is seen to be a loose reticulum with a moderate number of nuclei. There are numerous vessels and several of them have a thick wall of large, faintly staining cells; around some of the vessels there is a definite perivascular infiltration of small round cells and a few plasma cells. The leptomeninges in relation to this structure are slightly thickened and infiltrated. This abnormality is evidently a tubercle

and in this connection it may be noted that the microscopic examination of the lungs revealed a patch of acute tuberculosis.

The anterior horn cells of the more normal side show moderate chromatolysis. The posterior roots of the lumbar cord have more normal medullary sheaths than the anterior roots and on the side of the cord in which the tubercle is located the anterior roots are much paler than on the opposite side. The cell changes in the sacral cord are fairly marked. There is a great increase of glial fibers in the pyramidal tracts.

In the meninges of the brain there are brownish pigment bodies; these bodies are found in sections from the temporal lobe, occipital lobes, and especially in the region of the calcarine fissure and the gyrus fornicatus. There is also the glia infiltration of the cerebral meninges previously mentioned.

The pigment bodies are globular, though occasionally the pigment is found in a granular form; it is usually intracellular and is yellowish-brown in color. The cells containing the pigment are frequently large with the pigment to one side of the cell; the structure of many of these cells is that of "Abbauzellen." There is no constant relationship of the pigment or pigment containing cells to the blood vessels except in sections from the occipital lobe where there is evidence of increased accumulation around the vessels. Hemosiderin stains were negative but sections were positive for fat when stained with Scharlach R.

DISCUSSION

In comparing the two cases it is noted that there are points of similarity as well as of dissimilarity. The age of the first child was two years and ten months with the disease beginning at two years; the age of the second child was one year and five months; it is uncertain of the time of onset of the disease but lack of normal development is evident from the age of six months. In both cases the family history is negative and there is nothing significant in the past history.

Vision was involved in both children and hearing in the second and probably also in the first. The first child was definitely rigid when first seen and the deep reflexes were hyperactive at that time; later there was little rigidity and the knee jerks could not be obtained. The second child was spastic and had exaggerated deep reflexes except in the right leg where a flaccid paralysis developed while in the hospital. In neither case were there pathologic reflexes.

Both cases present in a general way a similar pathologic picture. Grossly there is a striking diffuse, symmetrical atrophy of the cerebral convolutions with wide but rather shallow sulci in brains which presented normally disposed fissuration. The fresh brains had an elastic firmness to the touch.

In both brains the microscopic picture consists of an enormous overgrowth of the glia tissue in the cortex and, to a much less extent, in the white matter of the brain, basal ganglia and brain stem and cord with marked involvement of the cortical nerve cells and to a less degree of the nerve cells of the basal ganglia, brain stem and spinal cord.

All types of glia cells are present from those with small nuclei and a faint margin of cytoplasm to large protoplasmic glia cells. The glia fibrils form a network with the glia nuclei and remaining nerve cells enmeshed in it; there is a marginal sclerosis with the fibrils extending into the pia and presenting the appearance, where the pia-arachnoid is a little pulled away from the cortex,

of raw cotton. The glia tissue is more dense around many of the vessels, and the processes of the glia cells can often be traced to the blood vessel. In both brains there is a formation of lacunae or a line of cleavage in the gray matter but it is much more marked in the first case. In the second case plaques simulating Alzheimers' foci are found throughout the cortex.

The first case shows definite chromatolysis of the cortical nerve cells and a normal appearing cell is rarely seen; in the second brain the cell changes are much more marked; in many sections from the cortex there is not a single nerve cell present and in other sections only cells showing advanced chromatolytic changes or only a "shadow" cell. Satellitosis and the so-called neuronphagia are present but nowhere marked. The nerve cells in basal ganglia, brain stem and spinal cord are progressively less involved.

The myelin sheaths of the first brain show little abnormality; the tangenital fibers are separated more than normally and there is some irregularity of the myelin sheaths in the radial fibers. In the second case the medullary sheaths show considerable involvement; macroscopically in the Weigert preparations no fibers are seen in the gyri and only a very faint staining in the area just beneath the convolutions; microscopically a few myelin sheaths are seen in the gyri; there is marked irregularity of the sheaths and occasionally there is a row of fat granule cells apparently in the place of a disintegrated nerve fiber.

The meninges are strikingly different in two cases. In the first case there is a thick boggy overgrowth of connective tissue and in the second case there is a thin delicate membrane associated with a marked external hydrocephalus. But they are similar in the absence of inflammatory reactions.

From the standpoint of the pathogenesis of the process it is evident that the gray matter of the cerebral cortex is primarily and most severely involved. It is probable that the condition represents a primary disease of the nerve cells with secondary glial hypertrophy; it is possible, however, that the glial proliferation is primary or the two processes may be intimately associated in their causal relations. There is no evidence of an inflammatory process and the lack of changes in the blood vessels preclude a vascular basis. It is most likely that the disease is an agenetic condition.

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WELCOME TO DALLAS*

By Dr. Edward F. Cooke, Houston, Texas

LAST year, my younger brother, Jack, was elected vice-president of the Texas Lumberman's Association, at Beaumont. Returning home, full of the honor conferred upon him, his train ran into a cow, damaged the cow, derailed the engine, and flung Jack from one end of the diner to the other. After he had been gathered out of the débris of chairs and tables, and had absorbed the contents of all available flasks, he opened his eyes and said, "If this is a part of the job, I resign right now." If, when I received my program last week, and was aware for the first time of the job assigned me, I had properly visualized this audience of brilliant men and wonderful women, I, in the words of my brother, would have resigned right now. I am not nor have I ever been what might be termed a tall man. Normally my height is about five feet, three and a half inches, in the morning, in my stocking feet, but ever since I entered this room I have been shrinking and shrinking, until now I can fully enter into all the feelings, and sympathize thoroughly with all the reactions of any ultramicroscopic filtrable virus.

As the program and the Toastmaster have informed you, I hail from the City of Houston, in this glorious State of Texas. I am very proud of my home town. I am glad to say that I can claim, without fear of successful contradiction, and prove my claim by our Chamber of Commerce and our local daily newspapers, that Houston is the largest inland *port* in the world, that she is the greatest *cotton* center in the world, that she is the greatest *oil* center in the world, that she is the greatest *lumber* center in the world, that she is the greatest *cattle* center in the world, that she is the greatest *railroad* center in the world, and she would be the greatest city in Texas, if it wasn't for Dallas. It might not be such a bad idea if some of you went on down to Houston and expressed your unbiased opinions as to whether or not Dallas is a bigger or better city than Houston. This is and has been a matter of much debate, pro and con; the pro has usually been on Houston's side, and the con on the side of Dallas. So we would be very glad of your assistance in settling the matter once for all. One suggestion, however. It would perhaps be wiser for you to await your return home before expressing your opinions.

You see this city of Dallas has a most infamous way of looking at the accomplishments of other cities, and then going them one better. San Antonio offers a six thousand dollar purse to the golfers. Dallas watches this for a while and then offers a ten thousand dollar purse. Last year Houston had the Admen's convention; last fall Dallas entertained the Southern Medical Association; not satisfied with that, she grabbed the American Medical As-

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists, Dallas, Texas, April 15, 16 and 17, 1926.

sociation, and still not satisfied, she topped that by grabbing the American Society of Clinical Pathologists. Galveston has her annual Splash Day, and oh, boy! San Antonio has her Annual Fruit, Flower and Vegetable Carnival, Waco has her annual Cotton Palace, Ft. Worth has her annual Live Stock Show. All of these are glorious and wonderful, but Dallas has her Annual State Fair, which is the biggest show of all. To the best of my knowledge, recollection, and belief, only two events have taken place in the Galveston-Houston district that Dallas has not emulated and surpassed. One of these was the hurricane of 1900, and the other was the hurricane of 1915.

You recall the old, old story of the mountaineer who, in his hospitality, told the visitor, "You're welcome, stranger. You're welcome to a potato, take two potatoes, take darn nigh all of them." In the same spirit of hospitality, having canvassed the State pretty thoroughly, on behalf of Galveston, on behalf of Houston, on behalf of San Antonio, Waco, and Ft. Worth, especially on behalf of Ft. Worth, I say to you, you are welcome to Dallas. Remember, however, if you accept this offer, it is entirely at your own risk. We cannot assume any responsibility, and you must undertake to remove the place from the State entirely. After that we do not care what you do with it.

Dallas is never satisfied. Like the magnet in the Gilbert and Sullivan opera:

> "A magnet hung in a hardware shop
> And all around was a glorious crop
> Of scissors and needles, knives and forks,
> But it wanted a Silver Churn."

You would think as you walk around these busy streets, noting the commerce carried on, looking at the magnificent buildings, reflecting upon all the rich agricultural country surrounding, that Dallas should be a happy contented city. But she is not, and the reason is that Dallas yearns to be a *port*. Her reasoning, of course, is perfectly sound and logical. Houston is a port and, therefore, Dallas can be a port also. There is another if rather insufficient reason. You may have noticed that Dallas is built on a bluff, a big bluff. If you stand on the edge of this bluff you can look down upon a little streak of moisture below. This streak is the Trinity River, which a little distance south of Dallas becomes quite a stream. Sometimes when we have a little rain up this way, this Trinity River gets so that you can see it distinctly with the naked eye. Whenever this happens, Dallas becomes a dreamer of dreams and a seer of visions, and again discusses the desirability of making the Trinity navigable. And do you know, it wouldn't surprise me if they do it some day.

But these Dallas folks are such terrible boasters. Nothing is small enough to escape their attentions along this line. I was enjoying a game of golf one day over one of their beautiful courses. I trust that you have had, or will have, an opportunity of agreeing with me that they have some very interesting golf courses here. While playing we heard three distinct shots

from either a rifle or revolver. I laughingly said, "Oh ho! another Dallas woman shooting her husband." One of the foursome, a Dallas lawyer, was just about to try and make a two foot putt, that should have been worth at least fifty cents to him. He turned to me with a look of outraged civic pride and said severely, "You are mistaken, Doctor; when a Dallas lady finds it necessary to shoot her husband, she only needs one shot."

They were having a regulation meeting here one day to discuss the question of making the Trinity River navigable, and several speakers had spoken about the advantages Dallas possessed, and lamented the fact that they were so far from the coast, admitting that in this one thing Houston had the advantage over them. If they just were as close to the Gulf of Mexico what an advantage it would be. Finally one gentleman, a visitor to Dallas, I think maybe he was from Rochester, Minn., got up and said that he was surprised to find out that there was even one thing that Dallas lacked, but that this lack could be overcome quite simply. It was just a question of a pipe-line to the coast he said, and then you gentlemen can have the Gulf of Mexico laving your courthouse steps by next morning, if you can just suck as hard as you can blow. The body was shipped home the next day.

This is not a booster's convention. You are not specially interested in any statistics as to the population of this, that or the other city, the number of miles of paved streets, or the amount of business done. If you were I could tell you of the variety of Texas, rolling prairie around Dallas, flat alluvial plains of the coastal region, the flat prairies with an elevation of six thousand feet of the Panhandle, the mountains of West Texas, and the forests of East Texas.

I could tell you a wonderful history of the State from the pirates of the Spanish Main to the pirates of the present day. I could tell you tales of the gradual coming of law and order. Of the Ranger Captain who, when sent to quell a riot, was asked on his arrival if he had been sent by himself alone, and his answer, "Well, you haven't got but one riot have you?" Of Captain Bill McDonald, of whom the Army Captain said, "He would charge Hell with one bucket of water." But these things would not particularly interest you. Remember though, as you wander around this city, that we are only showing you a sample. Dallas is not the only city we have in the State. We are glad to welcome you to a young and lusty Texas, to a city and State that has cast aside its swaddling clothes and, sensing something of future destiny, is surging upwards and onwards. There has always been a reason why the South has not made the commercial progress of other portions of the United States. There have always been two fears in the minds of those who have looked southward: one of these fears was of terrific heat; the other and more potent fear was of deadly disease. Yellow fever and malaria have been the greatest enemies the South has had to overcome. Today the greatest foe we have to face is the remembrance of these that still lingers in the minds of those who are casting keen eyes to the opportunities and rewards the South has to offer.

It would be quite interesting to chase the malarial phantom through this State. A visitor coming from, let us say, California would enter the State at El Paso. Dr. Waite, there, would assure him that they never have any malaria, but that they have an occasional case at San Antonio. Reaching San Antonio Dr. Stout would tell him that they have no malaria, but Houston has a good deal. Upon his arrival in Houston I would insist that malaria is so rare with us that when we get a case we call in our friends to see the slides, but that Beaumont is a regular hotbed of the disease. Dr. Thomson at Beaumont would sic the visitor on to Orange as a malarial locality, and Dr. Barr at Orange would assure him that he would have to go over the line into Louisiana, and then he would find plenty. So it would go all over the State, and we would find the same thing true in Louisiana. The fact of the matter is that to all intents and purposes yellow fever and malaria are things of the past. Science has triumphed over these diseases, and the glory goes largely to the men of the test tube and microscope. We are glad to have you down here to show you what your efforts are doing for our beloved Southland.

In regard to the other chimera, the heat and indolence of the South, look around you and see the truth. It has been shown that more and better work can be done if all the energy of the body be directed to that end, and none diverted to heat the plant. With the twin bogies of terriffic heat and prevalent disease relegated to the dim limbo of forgotten things, the South is about to come into her own industrially. None of us, no matter how optimistic we may be, have a sufficient vision to see the things that are about to come to pass in this State. We ourselves do not have any real conception of our resources, we do not fully realize even the vast area of Texas. One night two of my brothers and I left Houston by train. Each travelled in a different direction, two of us reached our destination by about seven the next morning; the third did not reach his until noon, and he was the only one to touch the Texas border.

The members of the medical profession only dimly, and the commercial and business men do not at all, realize the tremendous bearing on their financial welfare of the busy workers with culture media and pipette in a thousand and one laboratories over this country. These workers themselves have no conception of the impetus that their labors are giving to industry everywhere. Do you see Donald Ross peering into his microscope for weary weeks with sweat blinded eyes, until one day the demonstration of the sporozoites of malaria in the salivary glands of the mosquito? Do you hear the pean of joy swelling from his lips,

> "This day relenting God
> Has placed within my hand
> A wondrous thing, and God
> Be praised, at his command."

Do you find here any hint that he (Ross) has placed in the hands of a certain Col. Gorgas a wondrous tool that makes it possible to unite two

mighty oceans, each bearing its argosies of World Commerce? Do you find here any suggestion that when his own country should be in dire peril, and kin across the sea were hastening to the rescue, a certain Surgeon-General Gorgas could safely recommend sending thousands of men to training camps in a country where once the Spanish moss on the oak trees was called "Nature's Death-flag of Malaria"? Do you see any thought in his mind that he had become an empire builder, worthy to stand with his countryman Cecil Rhodes? No indeed, he was just a tired laboratory worker who had successfully finished his stunt, and what does he see?

"I know this little thing
A million lives shall save;
O Death where is thy sting,
Thy victory O Grave."

Therefore, as the men and women who are so largely responsible for the tremendous strides forward that our country is making, we are enthusiastic in welcoming you to the State of Texas and the City of Dallas, that you may see with your own eyes what you are accomplishing, and to express to you the South's debt of gratitude. You do not look upon yourselves as empire builders, you knights of Stains and Reactions, you diligent delvers into Nature's deepest secrets, but yours is this Empire, its power, its honor and its glory for ever and ever, world without end. Amen.

THE INTEGRATION OF HOSPITAL LABORATORY WORK*

By Philip Hillkowitz, M.D., Denver, Colo.

ACCORDING to a survey made by the American College of Surgeons there are close to one thousand hospitals in the United States and Canada each having a capacity of one hundred beds or over. All of these institutions, in order to comply with the minimum requirements of hospital standardization, presumably have a clinical laboratory equipped for carrying out the various routine examinations comprised under the term clinical pathology. In accordance with an official interpretation of the College these laboratories should be under the supervision of a competent clinical pathologist.

Inasmuch as over 85 per cent of these hospitals have received the approval of the American College of Surgeons, it would follow that all these clinical laboratories are conducting scientific investigations and helping the clinician in his diagnoses and treatment of disease. How far they approach the ideal desired, how closely the theoretic quantity and quality of proper laboratory supervision coincides with the actual state of affairs is beside the present discussion.

The fact remains that we have on this continent a grand army of laboratory workers with ample equipment capable of being marshalled and directed toward the most useful ends and unfolding undreamed of possibilities in the field of research and discovery.

At the present time the work of the individual clinical pathologist is more or less disjointed having no relation to the output of his fellows. Take the case of the average director of the laboratory in a medium-sized hospital. He exercises, to be sure, a most important and useful function in the conduct of the institution. His work in fact is nowadays indispensable. The routine urine and blood examinations throw a flood of light on the disease process and help the clinician in arriving at the diagnosis. The pathologist's interpretation of tissue findings is of far-reaching import in operative procedures. His presence at staff meetings, at the bedside, and in the operating room has a beneficent and stimulating influence on the internist and surgeon promoting exact and scientific methods in the diagnosis and treatment of disease.

Yet when all is said and done, there is often left the feeling of work half-done or uncompleted, of an aching void in solid accomplishment. At the end of the year when the hospital laboratory director takes stock of the results of his twelve months efforts, which by the way is not always done, he merely records the number of the various laboratory examinations performed—so many urinalyses, blood counts, Wassermanns, tissue examinations, etc. They form valuable statistics if properly evaluated; but what happens to them? The figures compiled are buried in the hospital archives. So far as their value

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists, Dallas, Texas, April 15, 16 and 17, 1926.

to the profession or to posterity is concerned they are as though hidden in an Egyptian tomb unless some future excavator will bring them out of their subterranean crypt.

The average pathologist is rather isolated from his fellows. Outside of the larger centers he may be the only one in his community and has to rely for stimulus and progress on the literature of this specialty in textbooks and in periodicals that more or less remotely deal with subjects of clinical pathology. In passing, it may not be amiss to point out the crying need of a special journal devoted entirely to the practical wants of the garden variety of clinical pathologist. This is said with all due deference to the excellent papers that are found in journals allied to our specialty.

A point of contact with his colleagues is reached through the annual conventions of the American Society of Clinical Pathologists which constitute a source of strong stimulation. One comes away from these meetings full of ambition and high resolve to emulate the noble example of our more gifted confreres who are contributing to the advancement of clinical pathology and the scientific practice of medicine. Once, however, we are back in our former habitat we lapse into our usual apathy and revert to the routine of our daily labors.

To but few of us it is vouchsafed to rise above the level of our surroundings, surmount all obstacles and make original investigations or a brilliant discovery. Even were we inclined to do original research, we know not where to begin. Bewildered by the multiplicity and complexity of problems to be solved, we cannot concentrate on one particular thing. Research often requires cooperation of several talents trained in one or more of the fundamental branches of science, which the individual laboratory worker has not mastered. The road to original research is long and arduous, requiring patience and perseverance in the unflagging pursuit of the goal.

The individual clinical pathologist, therefore, does not count for much in opening up new avenues for scientific advancement. But what we are unable to do as individuals, we can accomplish in the aggregate. Contemplate the potential possibilities of a thousand workers concentrating their attention on a few problems. If the plan is carefully laid out and each one has a well-defined task before him, the solution of any given problem will be easy. In other words, let us apply to hospital laboratory work the same principles that obtain in industry and which have made the United States the richest country in the world. Mass production has brought comforts to the many that were undreamed of in the past.

Mathematics and science being indissolubly linked together, I am using the term integration of laboratory work to connote the proper coordination of our scattered efforts into a harmonious whole for the benefit of mankind.

The clinical pathologists throughout the country would gladly embrace the opportunity to contribute to the common good. The spirit of research once engendered may kindle the spark of some latent genius who may be stimulated thereby to independent investigation and discovery.

The idea of utilizing the energy and talent of men engaged in the ap-

plied sciences to promote invention and progress is not at all original. It has been the subject of discussion at the sessions of the American Association for the Advancement of Science. The greater rewards and better compensation in the industries have drawn upon the best brains in the country depleting the ranks of investigators in pure science of the halls of learning. Similar steps are being taken by the larger industries to stimulate research even in the basic sciences by the laboratory workers, regardless of any direct bearing on their particular commercial project. How much more important and far-reaching if we apply the same procedure in the preservation of human life?

You will probably admit that the text of the sermon may be good but like all preachments not so easily susceptible of fulfillment in this cold prosaic world. I therefore have essayed to outline a few tentative suggestions whereby the American Society of Clinical Pathologists will be the central agency for initiating and directing such studies and investigation as it may deem most useful and practical, collecting the facts and data arising from this combined research work and deducing the logical conclusions therefrom.

In elucidation I may offer the following hints:

That a Committee on Research shall be appointed by the President of the American Society of Clinical Pathologists whose duty it shall be to draft plans for a collective investigation and study of such problems as they may deem useful and feasible. It shall draw up the details of the respective inquiries and allocate the work among as many clinical pathologists, be they members or not, as they may consider necessary, or apportion the particular line of investigation to such workers as have had special experience in this study. The Committee shall collate the data and results of the respective studies and submit a report upon it at a future meeting of the Society.

To facilitate the initiation of the project, the committee may first attack such problems as are within the technical capacity of the average clinical pathologist.

As to the studies that may thus be taken up in a wholesale manner, a vista of immense possibilities is opened up. I shall mention a few which seem to require an early solution.

1. A compilation and statistical survey of the annual reports of the hospital laboratories touching particularly the diagnosis of tissues removed at operation and the results of necropsy examinations.

Our present statistics of the prevalence of certain diseased conditions, based on the experience of particular clinics or hospitals are not necessarily true of the country as a whole. The clinical diagnosis as found in the archives of the boards of health are notoriously subject to error. The data based on careful gross and microscopic examination gathered by pathologists in hospitals throughout the country ought to present a true picture of the relative prevalence of disease in the different organs of the body.

2. The proper compilation of data as just outlined renders immediately necessary a uniform nomenclature of pathologic findings. At present we are

laboring to a great extent under the difficulty of not talking the same language pathologically. Various authors coin their own distinctive terminology to indicate identical conditions, leading to considerable confusion. This is particularly true of tumors. While no hindrance should be put in the way of original thought in the matter of nomenclature and admitting that the official pronouncement of the committee is likewise subject to fallacy, nevertheless the desirability of a commonly accepted and universally understood term is far better than the present babel of words to indicate pathologic diagnosis.

3. A foretaste of the problems with which such committee will have to grapple may be had from the following queries:

a. What constitutes chronic appendicitis (from a histologic standpoint).

b. By what criteria would you diagnose chronic amygdalitis?

c. Classification of goiter purely from the pathologic findings.

4. In addition the committee may gather the opinion of hospital laboratory directors on matters relating to the conduct of the laboratory, such as (a) What constitutes a routine laboratory examination for patients entering a hospital? (b) Should tonsils be examined microscopically? (c) Best methods of recording and filing laboratory findings.

5. Lastly may be mentioned the large field of research into such questions as the accuracy and suitability of various tests. This subject, I am gratified to find has already a place on our program for this year thanks to the stimulus of Dr. J. A. Kolmer. The study may be extended to include problems in pathologic anatomy and histology, such as grades of malignancy in cancer, the incidence of cancer in chronic mastitis, etc. The important status of the clinical pathologist in the modern hospital affords him excellent facilities for the study of follow-up records to help him answer these questions.

The suggestions sketched above present a bare outline of the scope of a committee on research and the lines of inquiry it may begin to pursue. If the American Society of Clinical Pathologists is to contribute its share to the advancement of medical science, which all of us devoutly wish to do, it has the opportunity to realize its aim by commandeering the collective efforts of its members to attain this end.

SUMMARY

1. Close to a thousand clinical laboratories are attached to as many hospitals of one hundred beds and over.

2. The clinical pathologist directing the laboratory is performing useful routine work which, however, is not evaluated or coordinated with the work of his colleagues.

3. The average hospital clinical pathologist either by nature or for lack of stimulus does little research work or original investigation.

4. Suggestion is made for the appointment by the American Society of Clinical Pathologists of a Committee of Research to draw up plans for series of collective studies assigning a definite task to the individual clinical pathologist.

5. A list of problems are enumerated which lend themselves to such integrated investigations.

DISCUSSION

Dr. Otto Lowy.—It seems to me that Dr. Hillkowitz's paper is a beautiful dream which I hope will come true. I believe this is a matter which should be properly taken up at our business meeting tomorrow and discussed and acted upon. I believe there is no association in the country that is so capable and fit to do the work that has been outlined by Dr. Hillkowitz. I hope this will be brought up.

TREPONEMATOSIS AS SEEN IN THE RURAL POPULATION OF HAITI*

By Commander C. S. Butler, (MC) U. S. Navy, Haiti, and Lieutenant E. Peterson, (MC) U. S. Navy, Haiti

IN THIS paper we are using the term "treponematosis" to include syphilis and the condition called yaws. We believe that the latter is simply one type of the protean disease, syphilis, and we will adduce evidence to substantiate this belief. Some of those who insist upon duality of viruses in treponematosis have little patience with those of us who believe that yaws and syphilis are identical.

Doctor Spittel, in his work on yaws, pays his respects to the ingenuity of those who differ in opinion from him and then proceeds to describe a disease which all the masters on the subject of syphilis since the time of Fracastoro put together could not differentiate from lues. The doctor makes his diagnosis on the frambesioma which he thinks is unlike anything else in its appearance and in its pathology. This, by the way, is not a fact, for the typical lesion of yaws is exactly like the condyloma of syphilis both histologically and in its general appearance. The condyloma is, to be sure, more often found on the moist parts while the frambesioma tends to appear on the unapposed areas of skin as well as on the moist parts, a fact observed by Pouppé-Desportes 125 years ago. The dualists, however, do not stop with the frambesioma. They also describe the circinate syphilide in the same kit with yaws and throw in, eventually, all the other skin lesions of syphilis for good measure, so that when one is through trying to dig a clinical picture of the entity yaws, out of any textbook on tropical medicine of the present day, he has a severe headache for his trouble and not much else.

Doctor Hugh Stannus,[8] in recent numbers of the *Tropical Diseases Bulletin*, gives a wonderful collation of the information contained in papers published on syphilis and yaws during the past few years. One cannot read this, however, without being impressed with two thoughts: First, with the truth of Osler's dictum, "Know syphilis in all its forms and manifestations and all other things clinical will be added unto you"; and second, with the wide

*From the Public Health Service of Haiti.

Read before the American Society of Clinical Pathologists, Dallas, Texas, April 15, 16 and 17, 1926.

divergence in conclusions reached when different medical investigators are looking at the same facts. It does not make any difference how good an investigator one happens to be, one nevertheless will often fit the facts to his own preconception of the question at hand. If there was ever a medical question which deserved to be looked at with historical perspective this question of yaws and syphilis is *that* one. If this historical approach had been used in studying the question in different parts of the world, advocates of duality would not ask the medical profession to believe that in such considerable population as that of Guam or Samoa, or anywhere else in the world for that matter, syphilis is absent. Such an error is simply due to the fact that the disease does not, in these places, present the picture of our preconception. The profession would not be asked to believe that yaws had died out from the negro population of the Southern United States where it had been introduced from Africa times without number and where the pabulum left nothing to be desired to propagate the disease true to form.

If yaws were not syphilis then the frambesioma should be a most common occurrence in the negro population of the South today. If yaws were not syphilis then our white people of the South should show the frambesioma, for this distinctive (?) lesion of the disease should breed true in the body of the recipient of the virus whether that recipient be a Negro, a Malay, or a Caucasian. Two hundred and seventy odd years ago Doctor Thomas Sydenham made the following statement regarding lues venerea (syphilis) and yaws:

> The lues venerea was introduced into Europe A.D. 1493, from the West Indies, it being, before that time, unknown even by name. Hence the disease is usually considered as endemic to the American colonies. In my mind, however, it is rather referable to the coast of Guinea, or to some portion of the Negro country thereabout. This I think because many of my countrymen have told me that, in slave ships, even before they have reached America, the disease breaks out, also that it breaks out with the natives in the country itself, and that independent of any previous unclean intercourse. Indeed, in some cases, it afflicts a whole family—men, women, and children. The disease that thus comes spontaneously is, in no respect, different from the true venereal lues. The symptoms, the pain, and the ulcers are the same—making allowance for the difference of climate only. The name, however, is different, the African disease is called the yaws.

With all the attempts at clinical and laboratory differentiation which have been made since Sydenham's time, his observations have never been disproved. It should have been the duty of those who advocate duality to disprove Sydenham's statements rather than to maneuver those who believe in unity into the defensive position of having to prove that yaws is something other than syphilis. As each item in the more exact knowledge of syphilis is unfolding itself, it is very quickly found that the same fact is true for so-called yaws.

We shall only mention a few of these:

1. The Treponema of so-called yaws was found to be identical with *T. pallidum*.
2. The serum reactions were found to be exactly parallel.
3. The clinical course of yaws is identical with that of syphilis.
4. The specifics of yaws are the same as those of syphilis.

5. The histopathology of the so-called yaws is identical with that of syphilis when the Treponema is alone in the lesion in question.

One of the writers of this paper (C. S. B.) has answered most of the minor objections brought up by the dualists. Reference in this connection is made to the following papers published upon this subject.[1, 2, 3]

In trying to build a clinical entity out of yaws it is always necessary to bear in mind the marked peculiarities which syphilis shows when operating upon a native race which race does not treat the condition. Failure to remember these peculiarities is the rock upon which many "maiden voyages" in tropical syphilology have come to grief. The man who knows his native syphilis loses all interest in one of the learned dissertations upon native rural syphilis which tries to make it produce aneurysms of the aorta, general paresis of the insane, tabes dorsalis, and, closer in to the primary, mucous patches and a high percentage of *demonstrable* roseolas and papulo-squamous syphilides. On the other hand the worse types of dermatitides, palmar and plantar lesions, and pustular skin lesions are the more common. Again the man who knows his tropical rural syphilis discounts immediately any paper in which the writer swallows the fallacy that in any human community on the earth syphilis is nonexistent. From an extended observation of many good medical men in action here in Haiti and elsewhere in the tropics we know that often they steer their mental ship into this narrow culdesac from which they are rarely able to put about and make for the open sea again. *Why* native tropical syphilis acts in this peculiar manner we do not know,—it just does. The question of strains of treponemas must be thrown out at once as the explanation. This little island of Haiti, not larger than the State of South Carolina, has been swapping treponemas with every race of man on the face of the earth for 434 years now and the Haitian melting pot has been able to take all "strains" and convert them into a type which gives the same symptomatic expression in the native Haitian, as it does in the Malay of Oceania and the Philippines or the Negro of equatorial Africa.

In the same manner the various "strains" of treponemas introduced into the Southern United States have been converted into the one type producing the orthodox syphilis. Treatment, clothing, and shoes have done it.

The marked influence of the surroundings upon the Treponema is well illustrated by Ramsay's observation in Assam. He found that florid yaws was only common among the plain dwellers in the warm season; in the cold season these people and the hill people at all seasons showed only condyloma-like lesions in the warm moist regions of the axilla, between the nates, etc., while with the return of the heat weather, or if the hill dweller came down to the hot plain, the disease again became florid.

The frambesioma itself is a typical example of how a treponematous lesion is affected by its environment. The attending foreign flora is responsible for the excrescent appearance of this lesion.

In the same manner external influence, such as trauma and superinfection, causes a plantar syphilide, occurring in an untreated and barefooted native, to take on the familiar appearance of the so-called "crab."

The fact that yaws occurs most often in childhood and that it *appears*

not to be transmitted from parent to child hereditarily is at first sight a serious drawback to the full acceptance of identity of viruses. It is necessary to remember in this connection: (a) that under primitive conditions of personal hygiene, syphilis is not one of the venereal diseases at all, but constitutes one of the exanthemas of childhood, and (b) that native hereditary syphilis and syphilis contracted early in life (syphilis insontium) not only develop an immunity, but when these individuals grow to sexual maturity their disease is either latent or tertiary. In either case there is much less chance of the disease carrying over to the offspring. D. C. McArthur[6] shows how unlike hereditary syphilis in the European is the native hereditary syphilis. While we do not agree with all McArthur says by any means, we are sure he is right in his contention that there is a great deal we do not know about syphilis hereditaria and that our conception of European hereditary syphilis cannot be taken to represent the condition as it occurs among native populations. (c) Good personal hygiene and especially adequate treatment convert this exanthem of "stone-age" childhood into a venereally acquired disease. If this is not true how are we to explain this epidemiologic monstrosity of a contagium capable of venereal transfer stopping at the outskirts of cities where the pabulum for its growth is greater and where personal contacts are multiplied to the *nth* power? How are we to explain the tropical and racial delimitation of a potentially venereal disease? No other contagious disease acts like this yaws if it *really* is limited to the country districts, and to the tropics, and to the dark races. How are we physicians to square ourselves with our conscience when we use a certain set of diagnostic standards to make a diagnosis of syphilis in a white man and the same features and standards to make a diagnosis of some other disease in a native? To our mind things that are equal to the same thing are equal to each other. (d) Much of the so-called yaws is due to late eruptions in hereditary syphilis.

Every textbook on tropical diseases makes the statement that yaws is inoculable upon syphilis and vice versa. This statement is made upon someone's pseudo exact ipse dixit and it has been copied by all writers for years with wonderful naïvete and punctilious accuracy. If this were true there would in all "yaws countries" be the usual rate of *venereal* syphilitic chancres. As a matter of fact it is rare to find primary syphilitic venereal lesions in rural Haiti. This is the experience of thirty odd men who know a primary lesion when they see it. Anyone of the Public Health Officers in the ten Public Health Districts of this Republic will subscribe to this statement. It represents the opinion of medical men who have examined and treated many, many thousands of cases of human treponematosis. Should a few antiquated contrary animal experiments be allowed to break down all this human evidence?

These ancient animal experiments are being revamped, however. During the last few years laboratory animals have been made to behave like human beings even in syphilis. An important discovery is that of Chesney and Kemp[4] who showed that a true immunity against syphilis develops in experimentally infected rabbits. The stumbling block of previous investigators has been the time element. These authors have shown that if a rabbit is treated with

arsphenamine and cured after the syphilitic infection has persisted for more than three months, the animal is then immune and cannot be reinfected with syphilis.

The criterion of cure and of absence of infection after reinoculation was the negative results in transfer of lymph nodes and tissue from various parts of the body.

This work must, of course, change our previous conception of the cause of resistance to reinoculation. Latent infection is not the only cause for this resistance; apparently a true immunity exists.

It is interesting to follow Nichols'[7] experiments after the work of Chesney and Kemp. Nichols has previously attempted to show that no cross-protection exists between yaws and syphilis. When Chesney and Kemp showed that the time element has an important bearing on the production of immunity in syphilis, Nichols repeated his experiments and concluded that the experimental suggestion is that long infection with yaws may partially protect against syphilis, and that a long course of untreated yaws in childhood may produce some true immunity to syphilis. Nichols adds, however, that the experimental protection of syphilis against itself is so much stronger than that of yaws against syphilis that the argument as to the identity of the two diseases fails.

In his experiments Nichols injected five rabbits with yaws and after a period of time varying from 142 to 376 days terminated the lesions with a sterilizing dose of arsphenamine. Within less than a month attempts were made to infect these animals with syphilis; two proved to be immune, two became infected with small chancres after a considerable time, and one rabbit had no local lesion but did have an infected gland. In a second series of three rabbits infected with yaws for 93, 225, and 91 days respectively the attempt to infect with syphilis succeeded only in the third rabbit which had had yaws for 91 days. Probably the time element had something to do with this result.

Voegtlin and Dyer[9] in a recent publication showed that of thirteen rabbits infected with syphilis and cured, none gave positive results after reinoculation with either syphilis or yaws.

These authors find it difficult to interpret the negative results of the inoculation of the treated rabbits with yaws. They quote the experiment of Neisser, Baermann and Halberstaedter (1906) in which these men succeeded in inoculating a monkey with yaws fifteen days after the appearance of a syphilitic chancre. Voegtlin and Dyer conclude that their treated rabbits, probably on account of the treatment, had developed a refractory state of inoculation with T. pertenue as far as the production of a local lesion is concerned. It seems rather odd to conclude that the treatment has such influence on pertenue and that some form of immunity or tissue resistance is responsible for the negative reaction in case of pallidum. Nichols (1911) and Kolle (1922) have shown that if reinoculation is performed within forty days both inoculations produce chancres. "In other words, persistent syphilitic infection, after a certain lapse of time, produces a condition of the scrotum which prevents the development of another chancre on reinoculation." Why should not Nichols' and Kolle's findings explain the experiment of Neisser and his

coworkers, and the findings of Chesney and Kemp with regard to syphilis in the rabbit explain the results of Voegtlin and Dyer with regard to yaws in the same animal?

The above findings by these various investigators are to our minds conclusive of the unity of viruses. These findings correlate the clinical observations of many investigators but place reports of "double" infections in mass in a very peculiar light.

We also believe that the failure of Jahnel and Lange[5] to infect general paralytics with yaws is another link in the chain that ties syphilis and yaws into one, in spite of the fact that Doctor Stannus says that no very definite conclusions can be drawn from these few experiments.

In order to make available the large experience in treponematosis which the Public Health Service of Haiti is accumulating, a questionnaire was addressed to three districts located:

1. In the North (Cape Haitian District).
2. In the middle portion of the Republic (Port-au-Prince).
3. In the South (Jacmel District).

These districts are in charge of the following Medical Officers of the United States Navy: (1) Lieut. Com. R. H. Laning; (2) Lieut. W. F. Kennedy; (3) Lieut. Com. R. P. Parsons.

A glance at a relief map of Haiti will show that this is a geographic cross-section of the Republic. The area covered in this study represents about one quarter of the Republic, that is to say, about 2,500 square miles. The total area of the Republic of Haiti is 10,204 square miles. It is a little smaller than the State of Maryland. On the map the 74th meridian of west longitude represents fairly accurately the boundary between the two Governments of the Island of Haiti, the Republic of Haiti, and the Republic of Santo Domingo. Recall the fact that no part of the Republic is more than a few miles from the sea and, therefore, from one of the many ancient port towns. Sixty to sixty-five miles would perhaps represent the greatest distance that one could be from the sea and still remain in the Republic of Haiti. Recall also the fact that no people on earth travel more within their little realm than the Haitians, both women and men. It is nothing for a company of Haitian women of the peon class to transport by mule, or horse and upon their heads market products from the little town of Saltrou on the Eastern end of the South coast of Haiti right over the highest mountain ridge of the Republic (Morne La Selle; the trail passes at a height of 6,200 feet) down off the North slope of this mountain into a desert, and then away off to the West through the Plain of the Cul-de-Sac to the city of Port-au-Prince. The distance is probably one hundred and fifty miles. The total of their market sales would perhaps be only a few gourdes. The women, however, have had "their hour" compensating for all the trouble and labor involved in the trip in meeting friends along the way and in the dicker and trade in the open market of Port-au-Prince. The same thing happens in every little mountain valley and gorge in the Republic, the adult population is in constant daily contact with the markets in the port towns. This has been going on for at least two centu-

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ries. Now let us recall the third fact which has bearing upon the epidemiology of Haitian treponematosis. Among the peon classes in the Republic sexual promiscuity is the rule. Formal wedlock is rarely entered into, but the children of looser unions are considered legitimate. With this remarkable state of affairs, is it not overwhelmingly suggestive that venereal chancres are so rarely noted? But this is the same thing noted by physicians in Guam, Samoa, and the Philippine Islands. There are two possible explanations for it: (1) That the population is so shot through with syphilitic virus that venereal syphilis can rarely find an infectable victim, and (2) that the venereal sores are overlooked. Our opinion is that the first is the correct interpretation, for not only do the skilled examiners of the Haitian Public Health Service, who are carefully examining for venereal chancres in every part of Haiti, rarely come across one, either in a man or a woman, but this also has been our experience in many different parts of the tropical world. This observation it seems to us should serve to settle negatively the assertion that yaws and syphilis are mutually superinoculable as far as human beings are considered.

The following is the questionnaire above referred to. We shall deal briefly with the answers received:

My dear Doctor:—

We are trying to gather evidence regarding syphilis and yaws and their possible relationship. We would appreciate very much if you would give us your observations according to the following scheme:

1. Distribution (towns or rural sections).
2. Age groups affected.
3. Immunity:
   Have you seen an individual with actual yaws or frank sequelae of yaws, such as crab, present a chancre?
4. What is your experience with congenital treponematosis? Is it common in your district?
   Are you able to differentiate between congenital syphilis and yaws in a young infant? If so, how? Do you believe that yaws may be congenital?
5. How do you differentiate between yaws and syphilis?
6. Have you by any chance seen the transition from secondary to tertiary yaws?

In answering the above questions please do not consult textbooks, but give impressions you have received from your own experience.

In order to illustrate the heavy incidence of infection, the following figures may be quoted from the January report of the Public Health Service of Haiti. During this month 30,976 out-patients were seen throughout the Republic; of this number 14,997 received injections (neoarsphenamine, sulpharsphenamine, or bismuth) for treponematosis.

Regarding question 1. There is complete agreement that the yaws syndrome is largely confined to rural districts and small villages.

2. Most any age may show the frambeside, but it is chiefly seen in children.

3. All agree upon the rarity of the venereal chancre.

4. All agree that congenital syphilis occurs in their respective districts especially in the larger towns. One physician states that he *never* has

seen the picture of congenital syphilis as observed in the United States. (Why has he not? There is admittedly plenty of syphilis in his district. Why do not the mothers abort and why do not the offspring show congenital deafness, interstitial keratitis, and Hutchinson's teeth? There's the rub—native congenital syphilis does not react like European any more than the adult varieties do.)

The differentiation between yaws and congenital syphilis in infants was stated to be nearly impossible where the history of extragenital primary lesion is not apparent. The answers to the question of yaws being congenital were very vague.

5. The differentiation between yaws and syphilis followed the lines of the ordinary textbook. All agreed, however, that in the third stage the most important differentiating point was the history.

6. None had seen it.

The important conclusion that can be drawn from the experience of the above men is that chancres in individuals with yaws, if they ever occur, are to say the least very rare. These men have seen only three cases of primary genital syphilitic sores in individuals who *apparently* gave histories of having had yaws. When the thousands of cases they have had under observation are taken into consideration the rarity of such an infection is evident.

In conclusion we would like to say that we have seen several examples demonstrating the truth of Hutchinson's statement that in his experience those Europeans who contracted yaws in the tropics returned home with syphilis.

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HEMOGLOBIN AND ERYTHROCYTES IN THE SOUTH*

By Leon S. Lippincott, M.D., Vicksburg, Mississippi

LAST year, before this society, I attempted to show that the average total and differential leucocyte counts in Mississippi differed somewhat from the textbook average normal standards. It was my belief that if studies could be carried out in various parts of the country as has been done in a few instances, we would either have to accept different standards for different localities, or, if these studies showed agreement, the usually taught standards would have to be revised. It was my belief that we have been too ready to accept standards set down in early studies as hard and fast rules for all time to come.

A further study of a series of hemoglobin determinations and erythrocyte counts is here presented. Most textbooks give the average normal hemoglobin percentage as 100,—this point varying somewhat with the method used for determination, and the average normal erythrocyte count as 5,000,000 for adult males and 4,500,000 for adult females.

The present series includes 1861 hemoglobin determinations and 1876 red counts made during the last six years on hospital and clinic patients, most of them as a part of routine laboratory examinations. The records were taken as they came without attempt to pick cases, except that hemoglobin readings below 60 per cent and red counts below 3,000,000 were not included. The findings from white and colored individuals were listed separately in order to determine any possible differences due to race.

The original hemoglobin readings were made with the Tallqvist scale because this method is less time-consuming than most of the others in use. I recognize the fact that an objection may be raised to the figures obtained because of the reputed inaccuracy of the Tallqvist method. However, a careful check with hemoglobin determinations made by the Newcomer colorimetric method in five hundred cases during the past year has shown Tallqvist readings, when the hemoglobin is above 50 per cent, to be more accurate and consistent than is ordinarily supposed. We found in the five hundred comparative readings that the Newcomer method gave an average percentage seven points higher than the Tallqvist scale. We believe that this higher reading is probably more nearly correct and in the tables given, we have added seven points to the Tallqvist readings.

The average hemoglobin determinations and erythrocyte counts in this series are as shown in Table I.

It will be noted that the hemoglobin, even when the Newcomer difference of seven points is added, and the erythrocyte counts are all lower than the

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists, Dallas, Texas, April 15, 16 and 17, 1926.

TABLE I

	HEMOGLOBIN (PER CENT)			ERYTHROCYTES	
	NUMBER CASES	TALLQVIST AVERAGE	CORRECTION PLUS 7	NUMBER	AVERAGE
White Males	716	78.20	85.20	639	4,539,000
White Females	725	74.71	81.71	779	4,253,000
Colored Males	214	76.28	83.28	232	4,428,000
Colored Females	206	74.26	81.26	226	4,140,000
Total	1861			1876	

usual textbook standards. I have to anticipate another possible objection to the figures obtained: that these findings are from hospital and clinic patients and are, therefore, not normals. While this is partially true, few included could be actually classed as anemia, and no case of anemia as shown by complete blood picture was considered. It was also found that when the color index is computed according to the usual standards, the diminution in hemoglobin while slightly more marked, in general corresponds in degree with the diminution in red cells. This is shown in Table II.

TABLE II

	COLOR INDICES	
	WITH TALLQVIST READING	WITH NEWCOMER READING
White Males	0.87	0.95
White Females	0.79	0.86
Colored Males	0.87	0.95
Colored Females	0.81	0.88

All of the color indices computed with the Newcomer standard for hemoglobin are slightly below one.

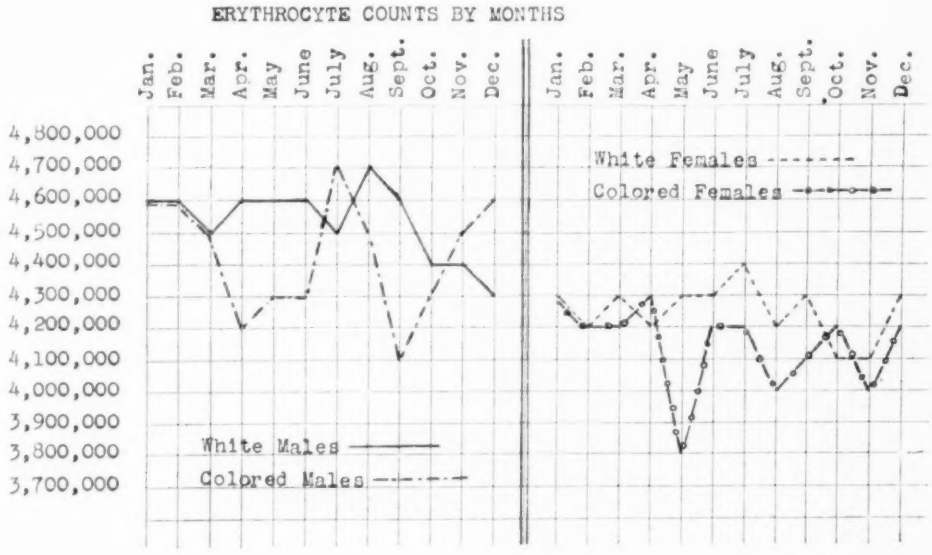
In this series, hemoglobin of 100 per cent was found in white males in 716 determinations but three times (0.42 per cent) by the Tallqvist standard and 14 times (1.96 per cent) by the Newcomer standard. In 214 determinations in colored males, hemoglobin of 100 per cent was not found at all by the Tallqvist standard and was found but three times (1.40 per cent) by the Newcomer standard. Hemoglobin of 100 per cent was not found at all in 725 determinations in white females and was found once only (0.48 per cent) in 206 determinations in colored females, this last being 100 per cent by both methods.

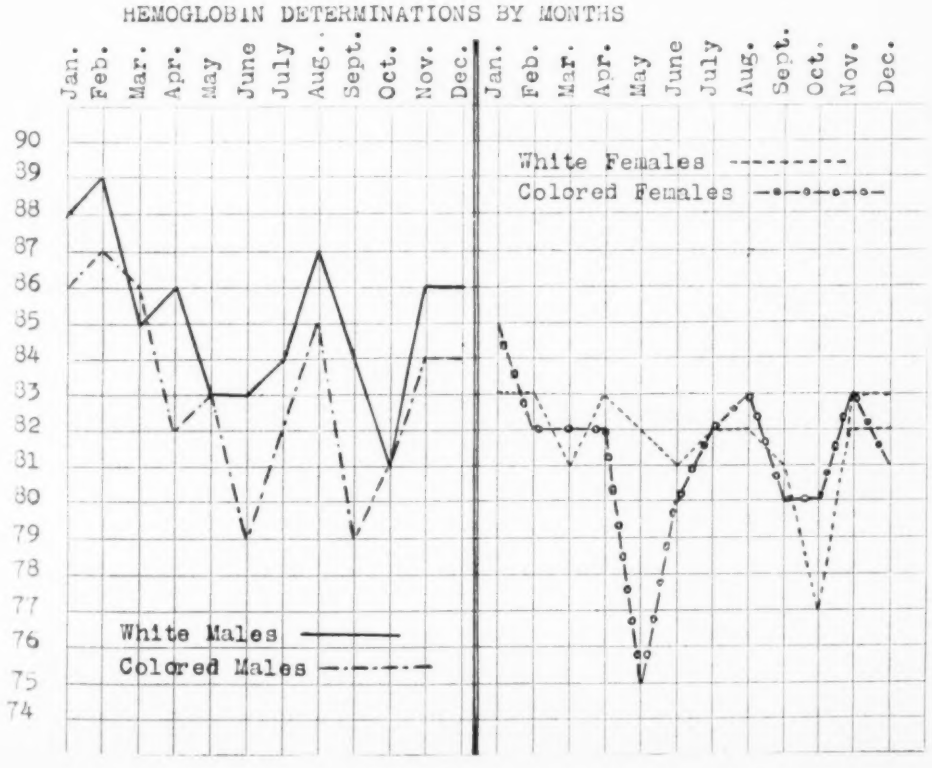
The standard 5,000,000 red cells was found in white males 117 times in 639 counts (18.31 per cent) and in colored males 32 times in 232 counts (13.79 per cent). The standard 4,500,000 red cells was found in white females 209 times in 779 counts (26.83 per cent) and in colored females 43 times in 226 counts (19.03 per cent).

Colored males show both hemoglobin and red cells slightly lower than is found in white males; and colored females show similar findings as compared with white females.

Working in the North some years ago, I do not recall that there was any reason to question the usual textbook normal standards for hemoglobin and erythrocytes. If these standards are correct for the North and the hemoglobin and erythrocyte counts are lower in the South as indicated by

the figures here given, then there must be a reason. Such a reason is not plainly evident in the literature that I have been able to find. It has occurred to me that the climate being warmer in the South, less oxidation is necessary to keep up the body temperature. As hemoglobin is the oxygen





carrier and the erythrocytes carry the hemoglobin, less of both of these blood components might be required.

In line with this reasoning the present series of determinations was listed by months to show any possible differences between hot and cold periods. The results are shown by the accompanying figures and in Table III.

In general it is shown that the hemoglobin is highest in January and February, which are our coldest months, with irregular reductions to October, followed by a rise in November and December. The colored females show a reduction in December, which is different from the others. This is probably not an accurate average finding. It is interesting to note that there is a rather sharp rise in August, one of our hottest months, in all classes.

The erythrocytes do not show the same changes as the hemoglobin, although there is considerable variation in the heights of the curves. All, with the exception of the colored females, show the highest points in July and August, and the colored females show a rise at that time.

The averages by seasons are given in Table III.

TABLE III

HEMOGLOBIN DETERMINATIONS BY SEASONS (PER CENT)

	DEC.–JAN. FEB.	MAR.–APR. MAY	JUN.–JUL. AUG.	SEPT.–OCT. NOV.	NOV. APR.	MAY OCT.
White Males	87.43	84.54	84.84	83.97	86.43	83.97
White Females	82.94	81.93	81.58	80.39	82.55	80.88
Colored Males	85.73	83.63	82.23	81.50	84.82	81.74
Colored Females	82.57	79.75	81.64	80.87	82.40	80.11

ERYTHROCYTE COUNTS BY SEASONS

White Males	4,506,000	4,555,000	4,600,000	4,493,000	4,508,000	4,669,000
White Females	4,272,000	4,281,000	4,305,000	4,157,000	4,241,000	4,266,000
Colored Males	4,610,000	4,341,000	4,397,000	4,263,000	4,503,000	4,352,000
Colored Females	4,277,000	4,104,000	4,134,000	4,093,000	4,194,000	4,085,000

Here we find the hemoglobin highest in December, January, and February, the coldest months, and lowest in September, October, and November, the end of the hot period. Also the hemoglobin is higher during the six months from November to April than from May to October.

TABLE IV

WHITE MALES

	HEMOGLOBIN (PER CENT)			ERYTHROCYTES	
	NUMBER	AVERAGE	PLUS 7	NUMBER	AVERAGE
January	94	81.06	88.06	96	4,639,000
February	76	81.64	88.64	68	4,608,000
March	38	77.50	84.50	35	4,505,000
April	60	78.83	85.83	31	4,600,000
May	39	76.28	83.28	29	4,560,000
June	45	76.33	83.33	26	4,587,000
July	64	77.42	84.42	47	4,494,000
August	68	79.85	86.85	72	4,719,000
September	57	77.46	84.46	52	4,636,000
October	58	74.48	81.48	61	4,418,000
November	53	78.96	85.96	55	4,426,000
December	64	78.59	85.59	67	4,271,000
Totals	716			639	

TABLE V

WHITE FEMALES

	HEMOGLOBIN (PER CENT)			ERYTHROCYTES	
	NUMBER	AVERAGE	PLUS 7	NUMBER	AVERAGE
January	69	76.23	83.23	68	4,275,000
February	94	75.93	82.83	97	4,233,000
March	56	73.95	80.95	58	4,281,000
April	60	75.58	82.58	66	4,235,000
May	57	75.26	82.26	58	4,326,000
June	53	73.77	80.77	63	4,325,000
July	61	74.51	81.51	65	4,425,000
August	54	75.46	82.46	63	4,165,000
September	61	74.26	81.26	66	4,261,000
October	47	70.00	77.00	53	4,094,000
November	60	75.92	82.92	65	4,115,000
December	53	75.66	82.66	57	4,306,000
Total	725			779	

TABLE VI

COLORED MALES

	HEMOGLOBIN (PER CENT)			ERYTHROCYTES	
	NUMBER	AVERAGE	PLUS 7	NUMBER	AVERAGE
January	31	78.87	85.87	30	4,632,000
February	27	80.00	87.00	27	4,580,000
March	27	78.89	85.89	28	4,529,000
April	15	75.00	82.00	14	4,210,000
May	5	76.00	83.00	9	4,283,000
June	19	72.37	79.37	18	4,278,000
July	13	75.00	82.00	13	4,682,000
August	6	78.33	85.33	13	4,535,000
September	15	72.33	79.33	16	4,056,000
October	16	74.38	81.38	20	4,277,000
November	25	76.80	83.80	28	4,456,000
December	15	77.33	84.33	16	4,618,000
Totals	214			232	

TABLE VII

COLORED FEMALES

	HEMOGLOBIN (PER CENT)			ERYTHROCYTES	
	NUMBER	AVERAGE	PLUS 7	NUMBER	AVERAGE
January	19	77.63	84.63	21	4,269,000
February	18	75.27	82.27	19	4,206,000
March	11	74.55	81.55	11	4,197,000
April	14	75.36	82.36	12	4,267,000
May	6	68.33	75.33	9	3,848,000
June	15	73.33	80.33	14	4,227,000
July	26	75.00	82.00	27	4,168,000
August	21	76.19	83.19	22	4,009,000
September	18	72.50	79.50	21	4,078,000
October	15	73.33	80.33	20	4,182,000
November	26	75.77	82.77	31	4,019,000
December	17	73.82	80.82	19	4,207,000
Totals	206			226	

The erythrocyte counts are highest in the period from June to August and lowest in the period from September to November in whites, both male and female; and highest in the period from December to February and lowest in the period from September to November in negroes, both male and female. Also in the six month periods, the red cells are higher from May to October in whites and from November to April in colored.

It is further interesting to note that of the hemoglobin determinations showing 100 per cent, 12 of the 14 in white males occurred in January and February; all three in colored males occurred in January and February; and the one in colored females occurred in December. The normal standard red counts found occurred more frequently in the colder seasons with the exception of those found in colored females, in whom season does not make much difference.

CONCLUSIONS

These studies indicate:

1. The normal average hemoglobin percentage and erythrocyte counts are lower in this locality (Mississippi) than the usually accepted normal standards.

2. The normal average hemoglobin percentage is approximately 84 per cent for males and 81 per cent for females, when the Newcomer method of determination is used.

3. The normal average erythrocyte count is approximately 4,500,000 for males and 4,200,000 for females.

4. Color indices average slightly below one.

5. In general, hemoglobin is highest in cold months of the year and lowest at the end of the hot period.

6. While erythrocyte counts do not change with the seasons as does the hemoglobin, most of the higher counts occur in the colder months.

7. There is a real need for a hemoglobin standard and a standard method of determination.

8. There is also a real need for the determination of what constitutes a normal erythrocyte count.

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DISCUSSION

Dr. C. L. Spohr.—During the discussion of a paper by Drs. Lindsay and Rice, and read at last year's meeting, points were brought out, favoring the use of reliable hemoglobinometers and the recording of the findings by grams per 100 c.c. of blood. I regret that the

author of today's paper has seen fit to make his hemoglobin determination by the Tallqvist method and to record them in per cent.

Dr. Herman Spitz.—I would like for Dr. Lippincott to tell us what investigations he has carried on in regard to the question of intestinal parasites in these cases, especially hookworm infestation. Also as regards chronic malaria. It would be interesting to know the incidence of these diseases during childhood and adolescence and what connection there is between these diseases and their accompanying malnutrition and the low values obtained by Dr. Lippincott.

Dr. E. F. Cooke.—I have not looked over my counts carefully since the program was received. I have rather the impression that there is a very marked degree of truth in the main in Dr. Lippincott's paper. Whether it is from being much further south than Nashville, Tennessee, or not I do not know. With the Tallqvist scale we run approximately 80 per cent for an average. I ordinarily use a Dare hemoglobinometer with which I am not satisfied; I am looking for something better. With the Tallqvist it has been my custom to add 10 per cent. I think that we do have a moderate decrease in the erythrocytes and hemoglobin in the South. I think I have noticed that it is. Sometimes people who have recently been in the mountains have a higher count and a higher hemoglobin than our people that have stayed home during the hot season. I do not think the difference between the negroes and the whites is of much importance. I do think that on the main Dr. Lippincott's contention is correct, that there is a diminution of erythrocytes and hemoglobin as you go South.

Dr. F. E. Sondern.—I am quite in sympathy with Dr. Lippincott's views. Of course I speak for a different part of the country and for a different class of people but the 100 per cent hemoglobin and the five million erythrocytes are distinctly the exception and not the rule. Our average figures are considerably below these as might be expected in the class of city-dwellers with which we have to deal.

Dr. Lippincott (closing).—I am very much interested in the whole question of blood counts. I agree with Dr. Spohr and accept his criticism; the hemoglobin should be given in grams per one hundred c.c. I did not do this because the majority of people understand the 100 per cent standard better. In regard to what Dr. Spitz said, we did not go into the history of each of these cases, they were merely routine laboratory cases. Last year there was an extremely low percentage of intestinal parasites. I am now looking over some figures on malaria and here again State Board statistics indicate too high an incidence. The iron is an important point. I had not thought about the metabolism; that may explain much of the difference noted. I want to thank you for your discussion.

LABORATORY METHODS

DROP-RECORDERS*

By O. S. Gibbs, Halifax, N. S.

SINCE every physiologist and pharmacologist is, will be, or has been, faced with the problem of recording the outflow or inflow of fluid, perhaps the following short review of the various types of available drop-recorders may be of some service. No claim is made, however, that this article is complete, especially as in most cases the origin of an instrument is obscure, and many modifications of any one type exist.

At least five principles are used in drop-recorders, namely:

Impulse
Weight
Expansion
Electrolytic
Displacement

apart from somewhat impractical methods such as photographic, or changes in electrical capacity.

The first type consists of some kind of a platform,[1] either balanced or held by a light spring, onto which the drop falls. Energy is thus imparted to the platform which is depressed, and thus either makes or breaks a circuit. This form of instrument is simple, which is the best that can be said for it. On the other hand it requires very careful adjustment, with a sufficient distance for the drop to fall, which is often very inconvenient, and it will not work satisfactorily if the viscosity of the fluid changes appreciably.

One instrument which had, however, an arm of about 20 cm. length responded fairly well, providing the circuit was broken by the falling drop.

Weight of the drop is utilized in several forms of instruments. The best of these has been devised by Condon.[2] In this instrument the drop is caught on a short counter-balanced platinum spiral; as it runs down this it momentarily adds its weight, thus causing it to tilt and close a contact. This instrument is simple and small, it works very well if properly adjusted. Unfortunately it fails if the fluid to be measured changes its viscosity to any extent. Without doubt it is a useful device for such work as urine flow in cats or rabbits. This instrument is often confused with the impulse variety, from which it is entirely distinct, since it works just as well from a bare clearance of the dropping tube, as from several centimeters height.

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Received for publication, November 26, 1926.

Another cruder and very simple form also devised by Condon (unpublished) is as illustrated in Fig. 1.

A is a tube held firmly in a clamp and connected by a piece of fine rubber tubing *B* and *C* which is a piece of thin glass tubing. As a drop forms on the end of *C* the extra weight bends down the tube which springs back to its original position when the drop falls off. In this way each drop is recorded. A well-made instrument of this type will function with all kinds of fluids, if somewhat imperfectly.

The third, or expansion type, has not been experimented with to any great extent. An example is illustrated in Fig. 2. *A* is a small half-cone bent out of thin metal. This is pivoted on a light arm, the counter end of which rests against a light spring contact *X*. The face of the cone rests against a piece

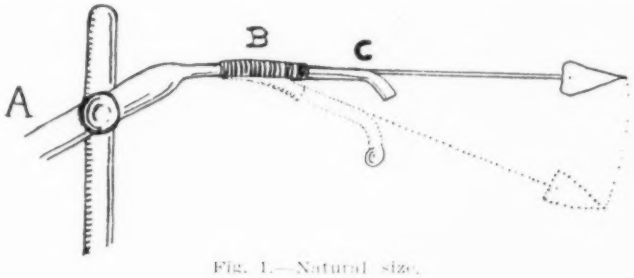

Fig. 1.—Natural size.

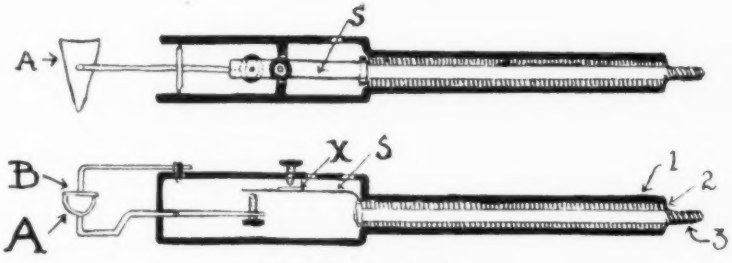

Fig. 2.—Natural size.

of flat metal *B* which is fastened to the frame of the instrument. As a drop falls in the cone it first fills up (2 to 3 drops), the next drop, in order to escape, forces the cone away from the plate and thus closes the contact *X*. Owing to surface tension effects a spring is not required in order to pull the cone back to the plate. This instrument has one advantage over most of the mechanical type for the moving parts are as it were on edge, in consequence of this they can be made much heavier than is usual, since very little power is required to move them. This renders the instrument less liable to accidental damage, beyond this, there is little to choose between it and the Condon type, since they both fail if the fluid changes its viscosity.

Expansion of a filling tube (vein) has been taken advantage of by Gesell[3] in his ingenious device, which no doubt could be made to function as an efficient drop-recorder. It is, however, somewhat complicated and also very expensive.

The fourth principle is that of causing the drop to fall between two electrodes, thus itself closing a circuit. Obviously this can only be successful if the fluid conducts electricity sufficiently well. Unfortunately for practical purposes urine alone, and even that not constantly, will work directly with this principle. It can be used with great success for measuring inflow, since most fluids injected intravenously are made up with Ringer or some such solution. For this, and any other purpose where the fluid is suitable, the apparatus shown in Fig. 3 works very well.

The principle used is that of the ordinary "sight-feed" lubricator, with the addition of the necessary electrodes *A* and *B*. *A* passes from the upper terminal to the dropping tube, and projects about 1 cm. downwards. *B* enters the chamber at right angles, but is bent down parallel to *A* at just sufficient distance from it that the down-falling drop touches both, but is not checked. In this way the circuit is momentarily completed. This instrument works

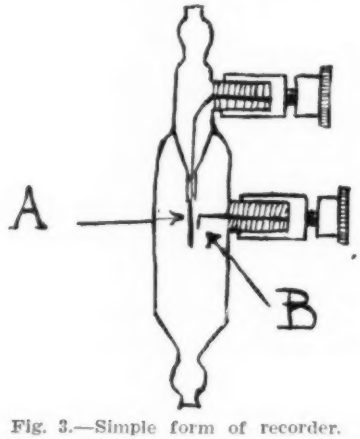

Fig. 3.—Simple form of recorder.

quite well up to 1000 drops per minute (about 30 c.c.), providing a good relay is inserted in the circuit, see tracing (Fig. 7).

The above apparatus is readily constructed from "Pyrex" tubing, but as will be easily appreciated it is almost impossible to make two dropping tubes exactly the same. As it is very useful to have two instruments working together it was decided to construct one in which the size of the drop could be altered. Two possibilities offered themselves, either increasing the dropping surface, or by elongating the dropping surface, thus causing the drop to break quicker. This latter procedure was adopted as appearing easier to make, and proved itself quite satisfactory. The final apparatus is as shown in Fig. 3-*A*, in which a fine threaded rod passes through the cap *M* to the dropping point, thus by simply screwing the rod down and increasing the length of the dropping surface, the drop breaks off sooner, and vice versa. A little extra complication is introduced since the electrode *B* must also be capable of vertical movement in order to be adjusted for the new dropping position. This was not difficult to accomplish as will be seen from the sketch Fig. 3-*A*.

Such an instrument as described above has many obvious advantages over the preceding ones, there being no mechanical parts to get out of order. Clearly its use is limited by the fact that many body fluids do not conduct electricity sufficiently well to work it successfully, even if a high voltage be used, and that in itself is apt to be disadvantageous. Also if the fluid changes its rate or viscosity, the drops will vary in size and thus impair the accuracy somewhat.

These facts have led to the construction of a very beautiful instrument by Hanike in which at least one very important advance was made. This in-

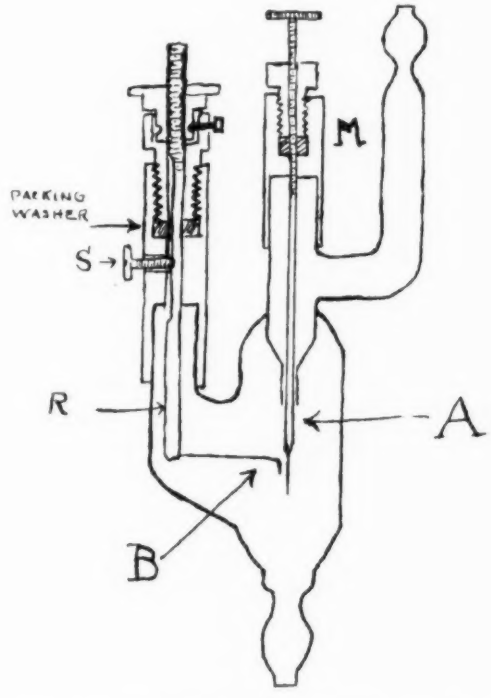

Fig. 3-A.—Form with drop adjustment. The terminals are not shown in sketch, they are soldered onto the side of the brass caps. Note the flat on the rod *R* onto which set-screw *S* bears. This is to prevent electrode *B* twisting.

strument utilizes the electrolytic principle, but in place of the body fluids acting directly they are used to displace 10 per cent sodium sulphate solution from another container. Secondly, the receiving electrode is connected to a slight minus pressure, thus ensuring a regular size of drop, and a clean contact break. Properly constructed this instrument has a very remarkable accuracy (it is claimed to $\frac{1}{200}$th of a c.c.) and unquestionably fulfils most of the requirements of a good recorder. Since it works primarily by displacement the viscosity of the fluid to be measured is of no importance, which is of moment in salivary work for which the instrument was first devised. Its size is not objectionable since fluid may be conveyed to the apparatus by a tube, which, especially if filled with fluid, does not impair its accuracy.

As this type has not been described in detail other than in Russian, apart from a cursory mention by Anrep[4] I venture to give the details of one form that works satisfactorily. Although this instrument is unquestionably somewhat complicated it is compensated for by ease of working. Fig. 4 is a photograph of the apparatus. *A* is the receiving vessel for the fluid to be measured, which for convenience has a drainage cock at the bottom as well as a shut-off cock at the top. The vessel is connected by means of a three-way tap to the sulphate vessel *B*. When in working position the fluid displaces air from *A* and forces sulphate up the tube *C* to the electrode *D*. This electrode is a concave platinum plate about 5 mm. in diameter with a small hole in the center. The drop forming on *D* eventually touches the lower or suction electrode *E* and at that moment makes contact, thus activating a relay. *E* is a slightly larger concave plate with a somewhat larger hole in the center, it is connected

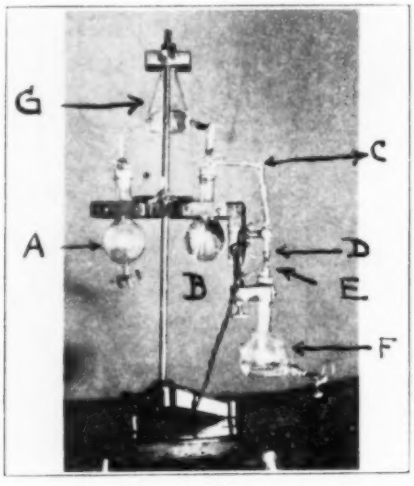

Fig. 4.—Complete apparatus with suction electrode for outflow records; 100 c.c. capacity.

through the flask *F* with a filter pump. Flask *G* is merely a reservoir of sulphate, which is used to fill the vessel *B* by means of the two three-way taps. *A* also can be emptied without disconnecting the animal, by means of its drainage cock and the three-way tap *T*. The sulphate solution can of course be used repeatedly. Where sudden changes of flow are expected it is advisable to fill the air-space with petroleum ether, or some such light insoluble fluid, since in an instrument of 100 c.c. capacity equilibrium takes a moment or so to establish.

Hanike's suction electrode is not applicable for measuring inflow, and the above type can only be used for recording outflow. If displacement principle be used in conjunction with the electrolytic type previously described even nonconducting, or viscous fluids can be accurately recorded. For this purpose, and for most others, the following type of instrument will satisfy all the usual requirements. (Fig. 5.)

A is the displacement chamber connected to a reservoir *B* by means of a

three-way cock which also serves as an outlet. Fluid is displaced from this chamber by means of the expansion of a fine rubber condom *C*. If the instrument is to be used as an inflow recorder (Fig. 5) sulphate solution (from a constant level device) is run into the condom, passing on its way through an electrolytic recorder of the type already described (Fig. 3). The expansion of the condom naturally forces out fluid from the chamber in exactly equal amount to that entering the condom, which will contain over 120 c.c. without stretching. For outflow records, on the other hand, the chamber is filled with sulphate solution, which on being displaced by the expanding condom which

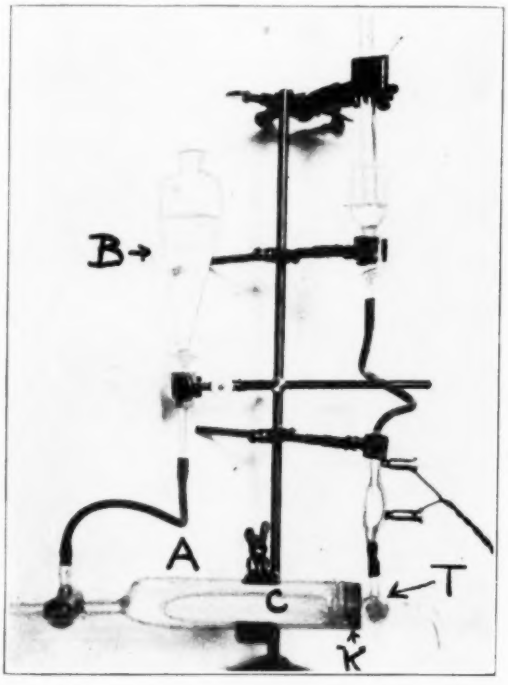

Fig. 5.—Arrangement for measuring inflow.

is connected to the animal runs through a recorder now connected with the tap *D*. (Fig. 6.)

The above form of displacement chamber is almost ideal, since relatively large quantities of fluid may be displaced with but little change in hydrostatic pressure. Furthermore it readily adapts itself for use with warm fluids. Since excreta may be required for analysis a special method is adopted for fastening the condom in order to facilitate its removal. This consists of turning down the end of the rubber cork *K* so that it is quite free from the walls of the chamber. On this a fairly deep groove is cut. The condom is pulled over this groove and fastened in position by a rubber band. In this way not only may the condom and its contents be readily removed but by leaving a clearance between it and the chamber walls it is not apt to become torn in pushing the cork well home. The practice of jamming the condom between

the cork and the chamber walls is not satisfactory as damage is so liable to occur to the delicate rubber, and furthermore the cork is apt to work loose owing to the stretching of the rubber. A second valuable point is that of extending the inlet tube right down to the very bottom of the condom, and making a series of holes along it. This is to prevent the fine rubber falling back in the inlet opening during the operation of filling the chamber (e.g., emptying the condom), which is done by means of the three-way tap at the inlet. (*T* Fig. 5.) Theoretically the fluid might just as easily empty a filled condom as vice versa, but in practice the way described is found to be more satisfactory.

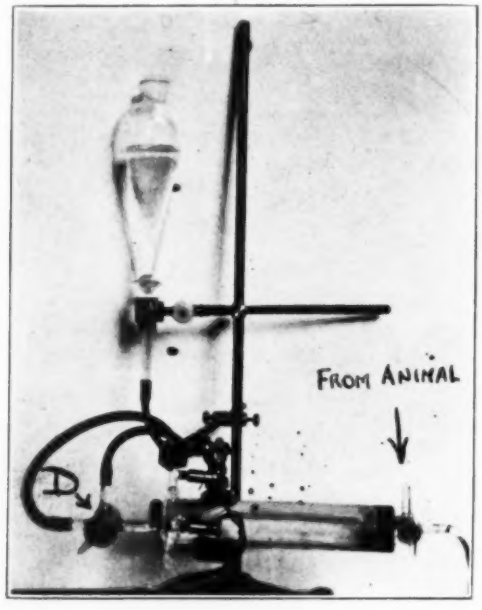

Fig. 6.—Arrangement for measuring outflow.

The displacement principle, of which but two types have been described, solves most of the difficulties associated with drop-recording, since the greatest fault of all drop-recorders is their failure to adapt themselves to changes in the fluid to be measured. Using displacement the actual liquid to be measured is relatively immaterial, since records can be made with the most suitable fluid for the particular instrument used. Thus in place of the electrodes shown in Fig. 3, one may easily replace any small mechanical drop-recorder, which if once adjusted properly should continue to function. Such a proceeding offers no advantage over the type described, since even a well made mechanical recorder requires much more attention than simply washing through with distilled water before and after use. For very exact outflow work there can be no question that Hanike's suction electrode is the most accurate and reliable at present obtainable.

Since practically all recorders of any moment are essentially delicate

electrical contacts, a little attention to the electrical side of the question amply repays some extra complication. Thus if in place of direct connection with the signal one uses a local battery and relay very much better results are obtained, and chances of failure greatly lessened. One might venture to say that no recorder, especially mechanical, will work unattended for long without the help of a relay, since the contacts, even if well designed, become dirty and corroded. A 1000 ohm spring type telephone relay, working off a 22.5 volt wireless battery, with a 1 m.f.d. condenser across its contacts has shown itself to be entirely satisfactory. (Actually two such relays are housed with the battery and condensers in a small box, since one has had frequent occasion to record both inflow and outflow at the same time.) The small extra trouble in connecting up is of little importance in view of the results obtained.

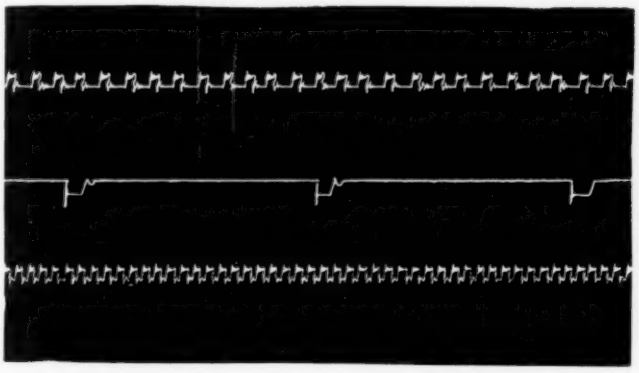

Fig. 7.—Upper and lower tracings drop records. Middle tracing time signal. Seconds recorded. The lower tracing is well over 1000 drops per minute.

Although the electrolytic type of recorder is the instrument of choice, some may prefer to use a mechanical type, may a plea be made on behalf of these much abused instruments. Mechanical drop-recorders are really instruments of great delicacy, and should, if satisfaction is to be expected, be made with at least the accuracy of a watch. In spite of this many of those obtainable have parts, even bearings, made out of twisted wire or some such rubbish. Such instruments are a source of annoyance until they are discarded. Bearings should be jewelled, failing that the pin-point type are probably the best. The moving parts of the recorder should not be used as one-half of the contact, since this means a bad connection at the bearings, unless a hair-spring be fitted. It is better that the moving arm merely activates a fixed contact as shown in Fig. 2.

SUMMARY

Several types of drop-recorders are described and discussed.

Methods of measuring both inflow and outflow are described.

Emphasis is laid on the displacement principle, especially used in connection with the electrolytic recorder.

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DESCRIPTION OF A NEW HYDROGEN-ION COLORIMETER

By R. B. H. Gradwohl,* M.D., St. Louis, Mo.

IT IS a matter of prime necessity for bacteriologists to adjust accurately the reaction of their culture media. Formerly, accurate quantitative measurements of hydrogen ions by the colorimetric method were dependent upon variable factors which many times led to erroneous readings. For example, buffer and indicator solutions were subject to deterioration, due to the growth of organisms. Indicators varied as to purity, and the intensity of their color changed markedly even in buffered solutions hermetically sealed. Research has gradually solved these troublesome problems, and with the advent of standardization of dyes, manufacturers have today placed on the market highly refined indicators. With materials and procedures standardized, it is now possible to offer accurate colorimeters for the measurement of hydrogen ions.

The electrometric method of determining the concentration of hydrogen ions is the ultimate standard of comparison, but since such a method is costly and requires a trained worker to follow accurately the various steps of the process, the colorimetric method holds sway. If buffered solutions are accurately checked by the electrometric method, growth of organisms prevented in the same, and the indicators of the highest purity used, then it is possible mechanically to approach closely by colorimetry the accuracy of the electrometric method.

The time honored method of determining the degree of acidity and alkalinity by titration has been discarded in most instances, due to its fallacies. Since the degree of acidity or alkalinity depends on the concentration in solution of H^+ ions, it necessarily follows that a satisfactory method of determining the degree of acidity or alkalinity must accurately measure quantitatively the H^+ ions in solution. This quantitative measurement is accomplished by determining the voltage produced in a solution containing hydrogen ions—each H^+ ion carrying an unvarying amount of electric energy. By means of a calibrated electric equipment we are able to measure accurately the amount of energy produced by H^+ ions in solution and subsequently to determine the number of these necessary to produce such energy.

In the colorimetric method of measuring hydrogen ions, buffer solutions play an important part. In general a buffer solution is one which is able to assimilate definite amounts of free acid or alkali without changing the con-

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centration of the $H^{\cdot}$ ions in solution. Since solutions absorb CO_2 from the air, which tends to make them acid, and also absorb alkalis from glassware, it is important that once a solution is checked electrometrically, it remains accurate.

Clark and Lubs' buffers meet the requirements. In certain of the buffers, however, molds are able to utilize the inorganic salts as food, and as a consequence the buffer after a certain period gradually becomes inaccurate. Hence it is of prime importance to render these buffers sterile and be assured they will stay so. This is a problem that has but recently been solved.

Fig. 1.—The Gradwohl colorimeter.

Now let us discuss the "buffer solutions." If all solutions were purely and entirely composed of known acids and alkalis, it would be comparatively simple to ascertain readily their P_H values. Such, however, is not the case. Most solutions which we desire to computate for acidity or alkalinity contain impurities and other substances beside their integral acid or alkali values. These impurities have a "buffer action" as noted by Clark which is "the resistance exhibited by a solution to change in P_H through the addition or loss of acid or alkali." In general the salt of any weak acid or weak base is a buffer salt. In making up an equipment to determine the P_H value of unknown solutions, we must work out a series of buffers which, with the addi-

tion of the proper indicator, give us a basis for comparison with unknown buffers, plus our known indicators.

Regarding indicators, it seems academic to note that they change in color when they are acted upon by solutions of different acidities or alkalinities. Clark and Lubs are responsible for the development of a number of indicators which have a wide range; that is, from extreme acidity on one hand to extreme alkalinity on the other. We have used the following indicators in our work in connection with this new equipment:

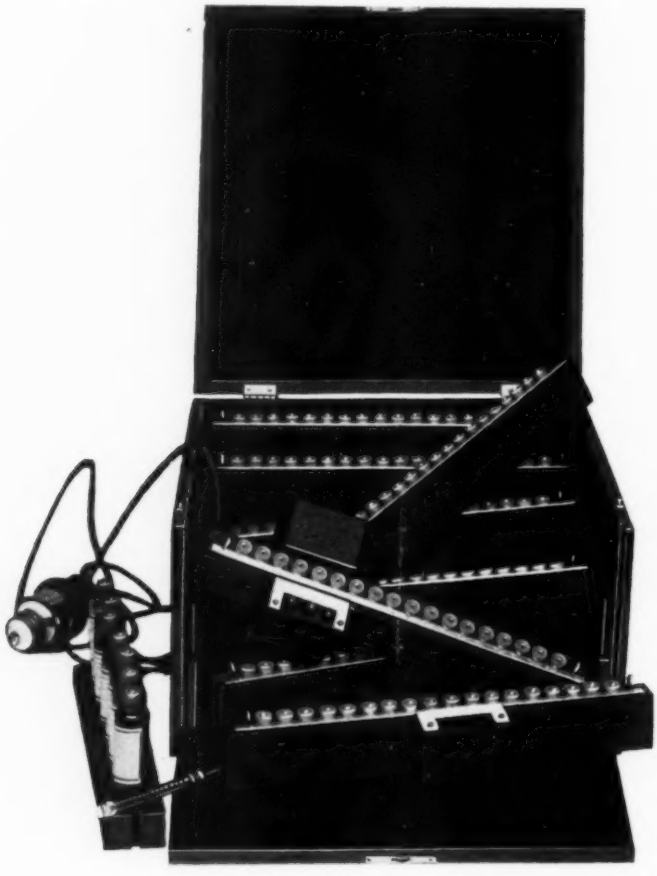

Fig. 2.—Equipment for test.

*Thymol blue	Ranging	from	1.2 to 2.8	changing	from	red	to yellow
Bromphenol blue	"	"	3.0 to 4.6	"	"	yellow	to blue
Bromcresol green	"	"	4.0 to 5.6	"	"	"	blue
Chlorphenol red	"	"	5.2 to 6.8	"	"	"	red
Bromthymol blue	"	"	6.0 to 7.6	"	"	"	blue
Phenol red	"	"	6.8 to 8.4	"	"	"	red
Cresol red	"	"	7.2 to 8.8	"	"	"	red
*Thymol blue	"	"	8.0 to 9.6	"	"	"	blue

*It will be noted that this indicator covers two hydrogen-ion ranges, one of extreme acidity, P_H 1.2 to 2.8, and the other of extreme alkalinity, P_H 8.0 to 9.6. The color change of the acid range is from red at P_H 1.2 to orange yellow at P_H 2.8. The color change of the alkaline range is from a greenish yellow at 8.0 to blue at P_H 9.6.

The equipment which we are about to describe consists of a box (Fig. 1) with a series of hermetically sealed special glass ampules containing buffers of known P_H value. The P_H value of each ampule will be found on the metal strip running along the top of the rows. In addition to this the indicator for each row is designated by initials on the end of the rack. Next, there is a series of indicator bottles properly labelled (Figs. 2 and 3). The illuminating box is furnished with an arrangement for sliding back and forth along any one of these rows of buffers which are taken from the box for comparative purposes, after a rough test has been made and the actual indicator selected.

Fig. 3.—Another view of equipment.

Before proceeding with the rough test, certain facts are to be borne in mind regarding P_H values. We know that the P_H value or rating of actual neutrality is 7.0. Any value above 7.0, such as 7.2 or 7.4, etc., denotes alkalinity. Any value below 7.0, such as 6.8 or 6.4, denotes acidity. We can change the P_H at will by adding acid or alkali as the case may be, to bring the value up or down. If you have a solution with a P_H value of 6.2, it is acid. You may want to make it 7.8. You do this by adding sufficient alkali. With these facts in mind we now proceed to carry out what is known as a "rough test," which is the effort to ascertain just what the hydrogen ion of the unknown actually is.

In a test tube place 5 c.c. of the unknown solution (Fig. 4) and add four or five drops of one of the indicators, preferably using the bromthymol blue first, for the reason that this has a P_H of 6.0 to 7.6 and therefore covers the neutral point P_H 7.0 as already described. In this way we can determine at once whether the solution is neutral, acid or alkaline. We know that bromthymol blue changes from yellow at 6.0 to deep blue at 7.6. If we add brom-

Fig. 4.—Manner of beginning "rough test."

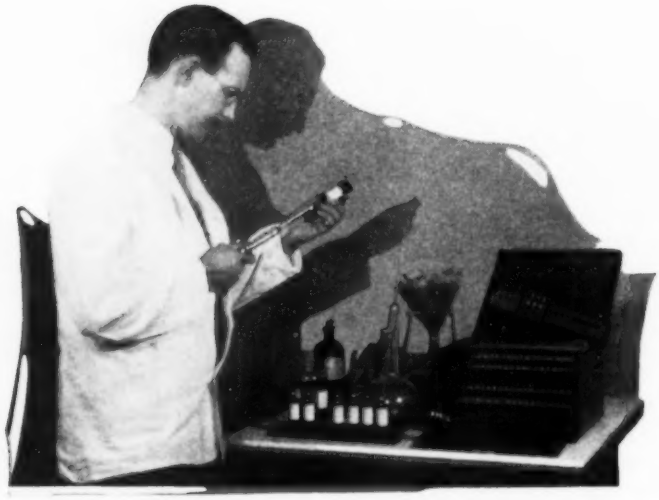

Fig. 5.—Method of using syringe and adaptor for withdrawal of indicator solution in exact quantity.

thymol blue and we obtain a color which is between yellow and deep blue, we know at once that the P_H value of this solution lies between 6.0 and 7.6 and that it is either neutral or very slightly acid or alkaline. In other words, it is on the border line of neutrality. If a yellow color is obtained by the addition of bromthymol blue we know at once that the P_H value is 6.0 or lower. Since the P_H value must be 6.0 or lower, we next proceed to use the indicator which covers the acid range of P_H 4.0 to 5.6, which is bromeresol

green. This must be added to the second test tube containing 5 c.c. of the unknown solution. Color change for this indicator is from yellow at 4.0 to deep blue at 5.6. Any color between yellow and deep blue means a P_H between 4.0 and 5.6. Proceed therefore to draw your conclusions as with the previous indicator. If on adding bromthymol blue indicator solution in the first test we obtained the deep blue color, the solution would be alkaline with a P_H value of 7.6 or even higher. To determine the higher value, use thymol blue which ranges from P_H 8.0 to 9.6. Having in this way obtained a rough idea of the approximate range of P_H value of the unknown, we proceed to make the actual test.

If you have found the range is between, say, 5.2 and 6.8, you place the standard amount of the unknown in a small test tube. Into this test tube

Fig. 6.—Adding indicator to unknown.

place 0.2 c.c. of indicator used in the rough test which gave you this range. This amount is withdrawn from the indicator bottle by means of the adaptor (Figs. 5 and 6) which the operator attaches to his own particular glass syringe (these adaptors may be obtained from Becton, Dickinson & Co., Rutherford, N. J.). Shake and place in the illuminating box. The other tubes containing the unknown solution without indicator are arranged on either side of the first tube containing the indicator, and the reading made against the unknown buffers of the identical indicator (Fig. 7). These buffers are taken from the box in the rack and identified by the initials on the end of the rack. When the color matches, the P_H value can be read from the known buffer solution.

For the benefit of those who have not used the hydrogen-ion method in adjusting the reaction of culture media we will give the following simple adjustment:

To adjust the reaction of culture media.—Let us assume that we are going to adjust the reaction of the culture medium to P_H 7.6. Choose as the indicators to be used phenol red, covering the range 6.6 to 8.2, and cresol red, covering the range of 7.2 to 8.8. Place 10 c.c. of the medium in a casserole. Add 10 drops of phenol red indicator. The color will become yellow if the medium is acid, red if the medium is alkaline, orange if neutral. If acid, add to the medium in the casserole N/10 NaOH drop by drop from a burette until the color changes from the yellow of the acid reaction to the red of P_H 7.6, as seen in the colorimeter. Read from the burette the number of c.c. N/10 NaOH used to adjust the reaction of 10 c.c. of medium. Measure the total quantity of culture medium made. Say you have 1000 c.c. of culture

Fig. 7.—Making color comparison.

medium on hand, it requires 100 times as much NaOH to neutralize 1000 c.c. of culture medium as it requires for 10 c.c. Multiply the number of c.c. of N/10 NaOH used by 100. One-tenth this quantity is the amount of N/1 NaOH to be used. The N/1 solution is used for final adjusting so that the total volume is not changed to any very great extent. Add to the culture medium the quantity of NaOH required and mix thoroughly.

Place 5 c.c. of the adjusted medium in each of two test tubes. To the first tube add 0.2 c.c. of phenol red indicator, to the second 0.2 cresol red indicator, mix, and compare in the colorimeter. The colors should match at 7.6 with both indicators. If the culture medium is still too acid, figure again the quantity of NaOH required to bring the reaction to the proper hydrogen-ion concentration. If it is too alkaline the reaction can be adjusted in the same way by using HCl instead of NaOH.

The particular advantages of this outfit described are as follows:

1. A series of standard buffer solutions prepared in a sterile manner which will resist deterioration.

2. Adequate buffers covering the entire range of acidity and alkalinity suitable for any kind of industrial or bacteriologic investigation.

3. The use of small test tubes with a minimum amount of indicator, thereby conserving the expense of purchasing fresh indicator solutions.

4. A uniform illumination which gives a standard and equal result at all times.

5. The mechanical arrangement of the racks with the illuminating box which permits one to adjust it quickly to rack after rack and perform the test in the minimum amount of time.

A RAPID METHOD FOR THE DETERMINATION OF CHLORIDES IN BLOOD OR URINE*

By S. L. Leiboff, New York City

A NUMBER of methods are described in the literature for the determination of chlorides in body fluids and tissues. All these methods, with but few exceptions, are based upon the same principle of precipitating the chlorine in the form of the insoluble silver chloride by means of an excess of silver nitrate in an acid solution. The excess of silver nitrate then being determined by volumetric means with a thiocyanate salt in the presence of a ferric salt which is used as an indicator.

Van Slyke and Donlevay,[2] and Austin and Van Slyke[3] determine the chlorides in blood by removing the proteins with pieric acid and determining the chlorine in the filtrate by titrating the excess silver nitrate with a standard potassium iodide solution using starch as an indicator.

Meyers and Short[4] also use the pieric acid filtrate of blood; they combine the three ingredients, silver nitrate, nitric acid, and ferric alum, in one solution, and after removing the silver choride by centrifugation, they determine the excess silver nitrate in the supernatant fluid with ammonium thiocyanate.

Whitehorn,[5] however, objects to the addition of nitric acid directly to the silver nitrate, as by doing so it may result in the mechanical enclosure of silver nitrate solution within the curds formed during the precipitation of the silver chloride, thus giving higher results. He uses the Folin-Wu[6] tungstic acid blood-filtrate in the Volhard-Harvey[1] method.

Later, Van Slyke[7] recommended a method whereby the blood proteins are destroyed by boiling with silver nitrate in a solution of concentrated nitric acid, and the excess of silver nitrate is titrated with standard thiocyanate salt and ferric alum.

The same objection may be raised to this method as to the one of Meyers and Short, with the additional disadvantage that an excess of nitric acid might exert a slight solvent action upon the silver chloride. The method is rather lengthy as it requires from one to two hours to digest serum and even longer periods to digest whole blood.

Isaac[8] described a colorimetric method for chlorides. He centrifuges the tungstic acid blood-filtrate of Folin-Wu[6] with magnesium carbonate and silver chromate and compares the color in the supernatant fluid against a known standard of the same color.

Dupray[9] improved this method by adding potassium iodide and sulphuric acid to the colored solution, thus obtaining a deeper color which is more suitable for colorimetric reading.

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Received for publication December 5, 1926.

Of the three main subdivisions of quantitative chemistry, viz., gravimetric, volumetric, and colorimetric, the gravimetric methods are usually the most accurate; but their technic is very lengthy and cumbersome, requiring great care and skill in their manipulation, so that their use in the analysis of clinical material is impractical, and in many cases, impossible on account of the exceedingly small amounts of material obtainable. The volumetric procedures, while not attaining as high a degree of accuracy in all cases as the gravimetric procedures, are sufficiently accurate provided great care is taken in the preparation and standardization of the solutions. They have the advantage over gravimetric procedures in that they are very rapid.

It is quite obvious that colorimetric procedures are the least accurate, for they are dependent upon a number of varying factors; such as the stability of the color, the proper adjustment of the colorimeter, the proper source of light, and most of all, upon the personal equation of the observer; for hardly any two individuals will obtain exactly the same reading on the same solution under the same conditions, nor will the same person duplicate his own reading each time.

In spite of the limitations placed upon colorimetric procedures, however, they find a great use in biologic chemistry because they furnish a method for the determination of very small amounts of substances which could not be determined by any other means. In the case of chloride determination, though, there is no advantage in using a colorimetric method, since chlorides are present in large amounts in blood and in urine, thus being very adaptable to volumetric determination.

Of the various volumetric procedures here described, the Volhard-Harvey[1] method seems the most adaptable for clinical work since it is the most rapid method and gives results equal to those obtained by other methods. It, however, has the disadvantage in that the end-point produced by the excess of silver nitrate with the ferric alum is not clearly defined. Also the cyanate solutions do not keep very well, so that Van Slyke recommends their restandardization at least once in two weeks.[7]

In the method which follows the cyanate is eliminated altogether, and the chlorine is determined directly with silver nitrate.

The principle of the method is as follows:

The solution containing chlorides is made neutral with calcium carbonate and titrated with a standard silver nitrate solution in the presence of sodium chromate which serves as an indicator, producing a red color when the end-point is reached.

Reagents Required:

1. Sodium chromate, a 0.25 per cent aqueous solution.
2. Calcium carbonate, a fine powder.
3. Standard sodium chloride, 0.1 per cent solution.
4. Standard silver nitrate solution, containing 1.452 gm. of silver nitrate in a liter of water.

Preparation of Pure Sodium Chloride:[10]

Since in this method the silver nitrate is standardized against sodium chloride, it is necessary that the latter salt should be of the highest purity. It may be prepared by the following simple technic:

Dissolve about 500 gm. of a good grade table salt in a liter of water and filter. Add concentrated HCl slowly until the chloride just begins to precipitate, and pass into the solution HCl gas until no more salt is being precipitated. When no more salt separates, filter through a Buchnell filter and drain by suction. Wash with about 200 c.c. of concentrated HCl solution in successive small portions, allowing to drain completely after each addition of the acid. Finally wash the salt with about 50 c.c. of water and test this wash-water for sulphates with $BaCl_2$. If sulphates are present continue the washing with HCl. Transfer the salt to a porcelain dish and heat until decrepitation ceases, cool in a desiccator for twenty-four hours and place in a well-stoppered bottle.

To prepare a 0.1 per cent NaCl solution dry a few grams of the salt in a desiccator to a constant weight, and dissolve one gram in a liter of water. This solution will contain one milligram NaCl in one c.c. of the solution.

PREPARATION OF STANDARD SILVER NITRATE SOLUTION

Weigh out about two grams of silver nitrate crystals and dissolve in a liter of water. This solution is more concentrated than is required. Dilute this solution so that two c.c. of silver nitrate solution will precipitate exactly 1 mg. of sodium chloride, as follows: Into a small beaker place 10 c.c. of the standard NaCl solution, add about 0.3 gm. $CaCO_3$ powder and 0.5 c.c. of the sodium chromate solution. Stir with a stirring rod and run in from a burette the silver nitrate solution until the first red color is obtained. The exact end-point can easily be recognized by preparing a control beaker containing 10 c.c. of water, 0.3 gm. $CaCO_3$, 0.5 c.c. of the chromate solution, and one drop of the silver nitrate solution delivered from the same burette; record the volume of the drop. The volume of the drop is to be subtracted from the volume of silver nitrate used to titrate the sodium chloride. To make up a liter of standard $AgNO_3$ solution use the following simple formula:

$$50 \times X = Y.$$

X Volume of $AgNO_3$ used to precipitate 10 c.c. of the NaCl.
Y Volume of $AgNO_3$ necessary to make up one liter of such strength that 2 c.c. will be equivalent to one c.c. of a 0.1 per cent NaCl solution.

For example, if it took 17.5 c.c. of silver nitrate to precipitate the 10 c.c. of the sodium chloride, then: $50 \times 17.5 = 875$. Thus if 875 c.c. of the silver nitrate is diluted with water to make up one liter, a solution of the proper strength is obtained. The standard solution should be kept in a dark bottle.

Procedure for Chlorides in Blood:

Place 5 c.c. of the tungstic acid filtrate of Folin-Wu[6] (0.5 gm. of blood) in a small beaker. In a similar beaker place 5 c.c. of water. This beaker is to be used as the control for the end-point. Add to each beaker about 0.3 gm. powdered $CaCO_3$ and 0.5 c.c. of the sodium chromate indicator. Add one

drop of the standard silver nitrate solution from a burette to the control and record the amount. Now run in the silver nitrate standard into the blood-filtrate until a change in color is produced similar to the one in the control. Subtract from the last reading the recorded reading of the control. This is the amount of silver nitrate used up to precipitate all the chlorine from the filtrate.

Calculation of Results:

$X \times 100 =$ mg. NaCl per 100 c.c. of blood.
$X =$ c.c. of $AgNO_3$ used (minus control).

Procedure for Chlorides in Urine:

Place 5 c.c. of urine into each of two small beakers. Add to each beaker about 0.3 gm. $CaCO_3$ and 0.5 c.c. of sodium chromate indicator. To one beaker, which is to be used as a control for the end-point, add one drop of the $AgNO_3$ standard from a burette and record the amount. Into the second beaker run in $AgNO_3$ standard until a change in color is produced similar to the one in the control. Subtract from the last reading the recorded reading of the control. This is the amount of silver nitrate used up to precipitate all the chlorine from the 5 c.c. of urine.

Calculation of Results:

$X \times 10 =$ mg. NaCl in 100 c.c. of urine.
$X =$ c.c. of $AgNO_3$ used (minus control).

(If the urine is alkaline acidify it with dilute acetic acid so that it is slightly acid to litmus paper before adding the $CaCO_3$.)

The accuracy of this method was checked up by adding known amounts of sodium chloride to various portions of a sample of blood and the total amounts of chlorine determined as described in the method. To a series of six flasks were added 2 c.c. of blood to each. This blood was previously found to contain 437 mg. of NaCl per 100 c.c. A 0.1 per cent solution of sodium chloride was added in increasing amounts, starting with 1 c.c. in the first flask and ending with 10 c.c. in the sixth flask. The proteins were then precipitated with tungstic acid, the bloods being diluted 1 to 10. Chlorine determinations were then done on 5 c.c. of each filtrate. As is shown in Table I the added chloride was recovered quantitatively:

TABLE I

SAMPLE	NaCl (0.1%) ADDED	TOTAL NaCl (THEORETICAL) IN 0.5 C.C. BLOOD	TOTAL NaCl FOUND
1	1 c.c.	2.435 mg.	2.438 mg.
2	2 c.c.	2.685 mg.	2.681 mg.
3	3 c.c.	2.935 mg.	2.937 mg.
4	5 c.c.	3.435 mg.	3.441 mg.
5	7 c.c.	3.935 mg.	3.939 mg.
6	10 c.c.	4.685 mg.	4.682 mg.

The method was also checked up gravimetrically on eight samples of urine. The gravimetric procedure was done as follows: 50 c.c. of urine was made acid with 1 c.c. of concentrated HNO_3 and an excess of silver nitrate solution was added with constant stirring. It was heated cautiously to boil-

ing until the precipitate settled, leaving a clear supernatant fluid. The AgCl precipitate was then washed, in previously weighed asbestos Gooch crucibles with hot water until 3 c.c. of the wash-water gave no precipitate with a drop of dilute HCl. It was then dried in an electric oven at about 120° C. to a constant weight.

TABLE II

SAMPLE OF URINE	GRAVIMETRIC METHOD PER CENT NaCl	PER CENT NaCl VOLUMETRIC METHOD
1	0.654	0.655
2	0.471	0.467
3	0.925	0.924
4	1.137	1.132
5	0.578	0.574
6	0.896	0.899
7	0.346	0.348
8	1.309	1.308

SUMMARY

A simple and rapid method is described for the determination of chlorides in blood and in urine. The chlorine is precipitated from solution by means of a standard silver nitrate solution in a medium kept neutral with calcium carbonate. The amount of silver nitrate used is shown by the red color produced by sodium chromate which is used as an indicator.

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A METHOD FOR ESTIMATING BLOOD PRESSURE IN RABBITS*

BY E. M. WATSON, M.D., LONDON, CANADA

ANY method for registering blood pressure in the intact animal is obviously beset with difficulties. Besides the technical obstacles involved in the actual estimation, the factors of fear, struggling, excitement and vasomotor instability on the part of the animal under experiment, tend to invalidate comparative studies. However, a method by which blood pressure changes in laboratory animals may be ascertained is desirable.

The principle of the method here described, as well as that of a device previously described by H. C. Anderson,[1] is that the central artery of the rabbit's ear is compressed by a rubber membrane, attached to a pressure chamber, upon an illuminated stage. The pressure chamber is connected with a mercury manometer and bulb for raising the pressure. The artery is

*From the Laboratory of Pathological Chemistry, University of Western Ontario Medical School.
Received for publication Oct. 27, 1926.

observed through a lens focused upon the stage and the pressure which is just sufficient to cause cessation of pulsation in the vessel is observed on the manometer.

In Fig. 1, *A* is a convex lens sealed to a piece of glass tubing, 15 mm. in diameter and of sufficient length to allow the lens to focus at a point just below the rubber membrane (*B*), which closes the lower end of the tube. From the pressure chamber so formed, a side tube leads to the manometer (part of a disused sphygmomanometer) and the pressure bulb. The stage (*C*) consists of the slightly convex end of a closed glass tube, 15 mm. in diameter and 15 mm. in length. This is sealed to a 6-volt, 21-candle power, tipless automobile lamp (*D*), about three-quarters of the surface of which is painted black. The light can be conveniently turned on and off by the switch on the base-board. The connection between the manometer and the pressure chamber consists of pure rubber pressure tubing.

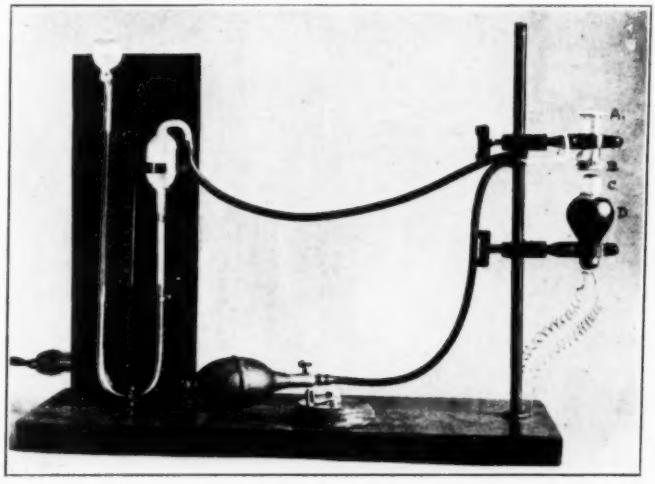

Fig. 1.

To use the instrument, the stage is adjusted to a convenient height and the rabbit's ear placed upon it in such a position that the central artery is directly over the stage. The pressure chamber is then lowered until the rubber membrane just touches the ear and is in line with the stage. If the light is switched on and the pressure gradually raised in the pressure chamber, the artery may be seen pulsating. When the pulsations cease, the pressure is read in millimeters of mercury on the manometer. For comparative readings, the same point on the artery should be used each time.

The blood pressure in the central artery of the ear of a normal rabbit, estimated by the method described above, ranges from 75 to 90 mm. of mercury. This is in accordance with the findings of Anderson[1] and of Shapiro and Seecof,[2] who used Anderson's method.

The apparatus described in this paper was made by Mr. A. Barber, my technical assistant, to whom credit is due.

REFERENCES

[1]Anderson, H. C.: Proc. Soc. Exp. Biol. and Med., 1923, xx, 295.
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TRANSACTIONS

Minutes of the Fifth Annual Convention, American Society of Clinical Pathologists—Dallas, Texas

THE proceedings were held in the ball-room of the Baker Hotel, Dallas, Texas, April 15, 16, and 17, 1926.

The convention was called to order by President Frederic E. Sondern. President Sondern appointed Dr. H. J. Corper Secretary pro tem.

Dr. Herman Spitz read the proposed changes in the constitution and by-laws to be adopted at the Executive Session.

Dr. Wm. H. Moursund delivered a welcome address to the Society.

President Sondern appointed the following members to serve as a Nominating Committee: Dr. Wm. H. Moursund, Dr. C. W. Maynard, and Dr. C. E. Roderick.

Dr. Paul Roth made a motion that a telegram be directed to Dr. Ward Burdick, Denver, Colorado, expressing to him the Society's best wishes for an early recovery. Motion carried.

The reading of papers on the regular scientific program followed:

"Hemoglobin and Erythrocytes in the South" by Dr. Leon S. Lippincott. Discussed by Dr. Carl L. Spohr, Dr. E. F. Cooke, Dr. Herman Spitz, Dr. Paul Roth, Dr. Frederic E. Sondern. Closed by Dr. Lippincott.

"A Combined Diluting and Staining Fluid for Differential Leucocyte Count in the Counting Chamber" by Dr. Daniel Nicholson. Discussed by Dr. A. H. Sanford, Dr. B. F. Stout, Dr. C. E. Roderick, Dr. F. W. Hartman, Dr. R. E. Myers, Dr. O. Lowy, Dr. Philip Hillkowitz. Closed by Dr. Nicholson.

"Sickle Cell Anemia" by Dr. G. S. Graham. Discussed by Dr. F. E. Sondern, Dr. B. F. Stout, Dr. Leon S. Lippincott. Closed by Dr. Graham.

"A Photographic Method for Counting Blood Cells" by Dr. A. H. Sanford. Discussed by Dr. H. J. Corper, and Dr. Wm. G. Exton. Closed by Dr. Sanford.

"A New Mechanical Principle for Automatic Pipettes and Blood Transfusion" by Dr. Frank W. Hartman. Discussed by Dr. C. E. Roderick, and Dr. A. H. Sanford.

The meeting was then adjourned.

Afternoon Session, April 15, 1926, 2 P.M.

Meeting was called to order by Dr. Sondern and scientific program continued.

"Determination of Sugar in Normal Urine" by Dr. Mark R. Everett. Discussed by Dr. Wm. G. Exton, and Dr. Wm. Taylor Cummins. Closed by Dr. Everett.

"The Glucose Tolerance Test" by Dr. W. B. Lewis. Discussed by Dr. Carl Spohr, Dr. Wm. G. Exton, Dr. A. H. Sanford, and Dr. F. W. Hartman. Closed by Dr. Lewis.

"A Study of the Pigment in Addison's Disease" by Dr. Carl L. Spohr, and Dr. Robert A. Moore. Discussed by Dr. Wm. G. Exton.

"Ochronosis" by Dr. Ernest Scott and Dr. Robert A. Moore. Paper read by Dr. Carl Spohr.

"Clinical Results with Pathogen" by Dr. Otto Lowy. Discussed by Dr. George T. Caldwell. Closed by Dr. Lowy.

"Pathology of Experimental Pyocyaneus Keratitis" by Dr. F. W. Hartman and Edna Jackson.

President F. E. Sondern appointed a pro tem Board of Censors consisting of Dr. A. H. Sanford, Dr. W. W. Coulter, Dr. H. J. Corper.

Meeting adjourned.

Morning Session, April 16, 1926, 9 A.M.

Scientific program continued.

"Frozen Sections, Their Place, Value, and Methods" by Dr. L. A. Turley. Discussed by Dr. Michael G. Wohl, Dr. Frank W. Hartman, Dr. F. A. Hecker, Dr. Philip Hillkowitz, Dr. Herman Spitz, Dr. Edward F. Cooke, and Dr. Leon S. Lippincott. Closed by Dr. L. A. Turley.

"Laboratory Examinations Necessary and Unnecessary" by Dr. George L. Schadt. Read by title.

"The Integration of Hospital Laboratory Work" by Dr. Philip Hillkowitz. Discussed by Dr. O. Lowy.

"The Cytomorphosis of the Tubercle Bacillus" by Dr. H. J. Corper. Discussed by Dr. Janet Caldwell. Closed by Dr. Corper.

"Oxygentherapy" by Dr. Paul Roth.

"The Treatment of One Hundred and Twenty-Five Cases of Acid Intoxication with Buffer Solutions" by Dr. F. A. Hecker.

Afternoon Session, April 16, 1926, 2 P.M.

Scientific program continued.

"Intestinal Amebiasis from the Pathologic Standpoint as Related to the Clinical, with Preliminary Report of X-Ray Studies of Early Cases" by Dr. J. M. Feder. Discussed by Dr. Isaac J. Jones, Dr. Kenneth M. Lynch, Dr. A. H. Sanford, Dr. W. S. Thomas, Dr. T. C. Terrel. Closed by Dr. Feder.

"The Hirsch-Abderhalden Test" by Dr. F. E. Sondern. Discussed by Dr. Wm. G. Exton, Dr. Otto Lowy, Dr. Mark R. Everett, and Dr. Michael G. Wohl. Closed by Dr. Sondern.

"Comparison of Kolmer and Kahn Tests" by Dr. C. E. Roderick. Discussed by Dr. T. C. Terrell, Dr. A. H. Sanford, Dr A. S. Giordano, Dr. F. A. Hecker, Dr. George T. Caldwell, Dr. J. M. Feder, Dr. F. W. Hartman, Dr. H. J. Corper, and Dr. B. F. Stout. Dr. Roderick closed the discussion.

Meeting was then adjourned.

Morning Session, April 17, 1926, 9 A.M.

Business session called to order by President Sondern.

The reading of the minutes of the last meeting was dispensed with.

Dr. Philip Hillkowitz gave the report of the Committee on Commercial Exhibits for Dr. Ward Burdick and a motion was made and carried that it be accepted.

The report of the Publication Committee was given by its Chairman, Dr. Philip Hillkowitz, who said that present arrangements for our official organ were not ideal and that eventually there would have to be a publication controlled entirely by the American Society of Clinical Pathologists of which the committee realizes fully the difficulties involved in the way of financing, managing, and editing; a proposition by Dr. Herman Spitz to be brought before the Society a little later. A motion was made that the report be accepted and the thanks of the Society be extended to the Publication Committee. Motion carried.

Report of the Committee on Methods of Transportation of Laboratory Specimens was made by Dr. Herman Spitz, Chairman, who said that no leaflet had been prepared owing to pressure of other business but that the post office department issued a set of rules governing this matter. These rules and regulations were received and published in the *Bulletin of the American Medical Association* of February, xxi, p. 62. A permit for sending and receiving specimens through the mails may be obtained from the postmaster. Motion made and carried that report be accepted.

Report of the Committee of Laboratory Standardization was made by President Sondern for Dr. Thaddeus Walker, Chairman of that Committee, who was absent. Dr. Sondern said that the Committee had been inactive because they deemed it advisable to wait and see what the American Medical Association did in the matter. Motion carried that report be adopted.

The report of the Executive Committee was given by Dr. Herman Spitz, Chairman, who gave the financial report and stated that the books of the Secretary were found to be correct. This report was accepted by motion of the Society.

The matter of a new code of ethics was taken up and the decision was reached that the present code was sufficient. Report accepted.

Dr. Spitz attended as representative of American Society of Clinical Pathologists, and read papers at two meetings of the American College of Surgeons. Report accepted.

The report on the Committee on State Laboratories by Dr. Herman Spitz was made; he gave various illustrations and records showing the tremendous amount of laboratory work done by State Laboratories in competition to practicing clinical pathologists. Discussion followed. Report of Executive Committee accepted by Society.

Dr. Herman Spitz read the revised constitution and by-laws and they were adopted.

Dr. H. J. Corper gave the report of the Service Bureau Committee for Dr. Ward Burdick, telling of the beginning of this feature of the Society. A motion was carried that this report be accepted and the thanks of the Society be extended to the Secretary for his work.

The matter of the Registration of Technicians was discussed and a motion carried that the President appoint a committee to investigate this matter and report to the Society at the next annual meeting.

The election of new members followed. Upon recommendation by the Board of Censors pro tem the followed were elected to active membership:

Dr. Oliver W. Lohr, Saginaw, Michigan.
Dr. Leonard W. Larson, Bismarck, N. D.
Dr. Charles F. Carter, Dallas, Texas.
Dr. C. Y. White, Philadelphia, Pa.
Dr. Kenneth M. Lynch, Dallas, Texas.
Dr. N. W. Loud, Colorado Springs, Colorado.
Dr. Oscar G. Costa, San Juan, Porto Rico.
Dr. Nathan Rosenthal, New York City, N. Y.
Dr. Wm. Taylor Cummins, San Francisco, California.
Dr. Wm. J. Muzzy, El Reno, Oklahoma.
Dr. Philip B. Matz, Washington, D. C.
Dr. Wm. McKee German, Grand Rapids, Michigan.
Dr. Joseph P. Garen, Olean, N. Y.
Dr. Reed Rockwood, Baltimore, Md.
Dr. C. H. Manlove, Portland, Oregon.
Dr. G. W. Millett, Portland, Oregon.
Dr. Carl Boettiger, Astoria, Long Island.

To associate membership:

Dr. Mark R. Everett, Norman, Oklahoma.
Dr. Isaac J. Jones, Little Rock, Arkansas.

Dr. John A. Kolmer suggested that a book of approved methods be published under the auspices of the American Society of Clinical Pathologists. A motion was made that a Research Committee be appointed by the President to investigate the question and study the advisability of such a publication and the ways and means of bringing this about, which committee was to have power to proceed. Motion carried.

The matter of a new journal, published and controlled entirely by the Society was opened. Dr. Herman Spitz presented a proposition for the consideration of the Society which was referred to the Executive Committee with power to act.

It was moved that a Committee be appointed by the President to investigate the matter of bringing the views of the Society forward by various ethical methods to be determined by this committee which was given power in the matter.

The election of officers for the ensuing year resulted as follows:

President Elect:	Dr. A. H. Sanford, Rochester, Minnesota.
Vice-President:	Dr. J. H. Black, Dallas, Texas.
Secretary-Treasurer:	Dr. Ward Burdick, Denver, Colorado.

Executive Committee:

Dr. Philip Hillkowitz, Chairman, Denver, Colo.
Dr. Wm. Carpenter MacCarty, Rochester, Minn.
Dr. F. W. Hartman, Detroit, Mich.
Dr. John A. Kolmer, Philadelphia, Pa.
Dr. W. W. Coulter, Houston, Texas.
Dr. Herman Spitz, Nashville, Tennessee.

Board of Censors:

Dr. George Ives, Chairman, St. Louis, Missouri.
Dr. Ernst A. Victors, San Francisco, California.
Dr. Paul Roth, Battle Creek, Mich.
Dr. W. F. Thomson, Beaumont, Texas.
Dr. Robert A. Kilduffe, Atlantic City, New Jersey.
Dr. H. R. Mills, Tampa, Florida.

Dr. Wm. G. Exton, President for 1926-7 was escorted to the Chair and the meeting was adjourned.

DR. WARD BURDICK, SECRETARY.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Clinical and Experimental

PERTUSSIS: Significance of the Blood Chemical Changes in Pertussis, Regan, J. C., and Tolstouhov, A. Jour. Am. Med. Assn., April 10, 1926, lxxxvii, 1166.

A study of 111 cases ranging in age from four months to twelve years, 78 per cent being over three years of age.

A total of 682 blood chemical analyses performed in cases of pertussis have given the following results:

1. There is a diminution of the total inorganic phosphorus associated with a lowering of the hydrogen-ion concentration of the blood, while the plasma bicarbonate remains within normal limits.

2. These changes occur early in the disease, appearing in the case of the inorganic phosphorus in the catarrhal stage.

3. Both alterations are well developed, especially the change in phosphorus, during the first few weeks of the paroxysms, and show a certain degree of parellelism in their course which signifies a close interrelation.

4. In moderate and severe cases treated with alkalis, the inorganic phosphorus rises steadily from the third week, while in untreated cases of the mild type, the rise does not begin until the sixth week. The same is true in a less decided way of a P_H value before, as compared to those during and after, treatment.

5. The diminution of inorganic phosphorus bears no relation to age, but only to the stage of the disease, and for reasons mentioned in the text has no underlying rachitic basis.

6. The calcium content, while exhibiting slight mobility, as the result, possibly, of shifting of calcium in connection with the characteristic phosphorus and P_H alterations, has no constant alterations of a distinct type.

7. These changes indicate an acidosis of an uncompensated type (type 6, Van Slyke), which has as a cause the accumulation or increased concentration of free carbon dioxide in the blood. This laboratory observation is easily correlated with several of the symptoms so prominent in pertussis—the paroxysms, the vomiting, parenchymatous emphysema and convulsions.

8. The vomiting of the disease may be a compensatory mechanism adopted by the body to eliminate acid in an attempt to maintain a normal acid base balance.

9. This contention of an uncompensated acidosis is further substantiated by the effects of the disease of alkali therapy.

10. Alkalis administered early appear usually to abort the disease, and associated with the cure is a rapid rise of inorganic phosphorus and a change in P_H of the blood, while, if given late, cure supervenes in a relatively short period.

Conclusions: There occurs in pertussis an uncompensated acidosis, which is intimately connected with the pathogenesis of the paroxysms. If the acid base unbalance is corrected, the clinical symptoms are quickly ameliorated, and the organism returns to normal.

EDEMA: The Cutaneous Test for a Hydrophilic Condition, Labbe, M. Presse Med., Paris, May 22, 1926, xxxiv, 641.

When small quantities of physiologic salt solution are injected into the derma of an edematous patient, the small blister thus produced disappears in a few minutes, while it remains for more than an hour in a normal individual. Interesting experiments have been performed with this fact as a basis.

The injection cannot be considered as properly effected unless it produces a small round local elevation, snow-white and showing plainly the pores of the skin. A small erythematous zone surrounds this elevation.

The time elapsing before the disappearance of this blister varies for different pathologic conditions, and its evaluation throws light on the more or less accentuated hydrophilic condition of the tissues and humors.

The slight traumatic elevation produced by the introduction of the needle must be distinguished from that due to the saline injection. The former may persist after the latter has completely disappeared.

It has been noted that in edematous patients, the more accentuated the edema, the more rapid the disappearance of the blister, and inversely. In incipient edema a reduced period of disappearance precedes clinical signs of edema by several days.

The Barthelemy syringe is to be recommended, as well as the use of as fine a needle as possible.

In ascitic patients, the time of disappearance diminishes as the ascitic fluid increases. The shorter the period of disappearance, the greater the increase in the ascites.

After serious hemorrhage the time of disappearance increases with the reestablishment of the bulk of the blood stream. Thus the diminution of the time of disappearance produced by intradermal injection of a saline solution indicates a thirst for water in the tissues. This test has an important bearing on the study of the hydrophilic condition of the tissues and humors, and hence that of the pathogenicity of the edema present.

Edematous plasma was injected into three normal subjects and the resulting blister did not disappear before the expiration of the time previously required for the disappearance of the blister following injection of the salt solution; that is, from one to one and one-half hours. When injected into a patient suffering from Laennec's cirrhosis with ascites, the time of disappearance was reduced to fifteen minutes.

Upon injection of normal plasma into three normal subjects, the time of absorption varied from fifteen to seventy-five minutes.

Thus the test gave the same results whether made with normal plasma, edematous plasma or physiologic salt solution, showing that the rapidity of the time of absorption is independent of the liquid injected, and depends entirely on the hydrophilic powers of the tissues and humors.

Diabetic patients, even where there are no clinical indications of edema, have a particular tendency to retain water in their tissues, nor does insulin treatment restrict this predisposition. Many diabetic patients are in a predematous state, evidenced by the peculiarly elastic and soft consistency of the tissues noticeable upon palpation. The cutaneous test corroborates such evidence as the time of disappearance is always noticeably shortened.

BLOOD SEDIMENTATION: The Relation of the Erythrocyte Sedimentation Reaction to the Ability of Flocculation of the Plasma and Serum, Rubin, E. H. Arch. Int. Med., June, 1926, xxvii, No. 6, p. 848.

Depending on the toxicity of a disease process, a proportional increase in the erythrocyte sedimentation reaction and the ability of flocculation of the plasma and serum, as determined by the Frisch and Starlinger, Gerloczy and Matefy reactions, was found. The Daranyi reaction was negative in forty cases studied.

Because of its greater simplicity, accuracy and wider range of readings the sedimentation reaction seemed the most practical test to use clinically.

BLOOD SUGAR: A Comparison of the Folin-Wu and the New Benedict Method for Sugar in Blood and Cerebrospinal Fluid, Lyttel, J. D., and Hearn, J. E. Jour. Biol. Chem., June, 1926, lxviii, No. 3, p. 751.

Simultaneous blood and cerebrospinal fluid sugar determinations were made on twenty-six cases by the Folin-Wu method and by Benedict's new method.

In the majority of cases the Folin-Wu method gave higher values than did the Benedict method.

In the blood the average difference by the two methods was 12.4 mg., with 13.8 per cent of the cases showing practically no difference.

The nonprotein nitrogen content of the blood had no relation to differences in sugar content by the two methods.

In the cerebrospinal fluid the average difference was 3.1 mg. with 52.6 per cent of the cases showing practically no difference.

The interfering substance or substances are not present in the cerebrospinal fluid so constantly or in such large amounts as in the blood.

The protein and nonprotein nitrogen content of the cerebrospinal fluid has no relation to differences in sugar content by the two methods.

EDEMA: Intradermal Salt Solution Test in Normal and Toxemic Pregnancies, Lash, A. F. Surg., Gynec. and Obst., July, 1926, p. 40.

The technic of McClure and Aldrich was employed as follows: 0.2 c.c. of an 0.8 per cent aqueous solution of sodium chloride was injected intracutaneously under aseptic conditions. A duplicate injection was made about 2 centimeters from the first, as the disappearance of the depression between the two wheals was an aid in determining the end point. The flexor surface of the forearm and the medial surface of the leg were the sites selected for the injections. The disappearance time was the time taken for the elevations or wheals to disappear as determined by palpation. The frequency of observations depended on the character and course of the case; thus, in the normal pregnant women one careful test was considered sufficient, while in the abnormal, tests were performed at intervals of several hours, days, or weeks. In the normal women a five to ten minute variation in disappearance time was considered of no significance as the end point was not always well defined in this group, although definite in the toxemic women.

A normal group of 47 pregnancies was studied (20 white and 27 colored; 15-36 years of age; 26 I-para; 21 multiparae; urine and blood pressure normal).

A group of toxemic cases was also studied (17 with convulsions; 29 without).

The following conclusions were drawn:

The disappearance time of intradermally injected salt solution in normal pregnant women is longer in the negro than in the white. This racial difference can possibly be explained by the thicker skin in the negro (Unna, 8).

The women with the toxemias of pregnancy show definitely decreased disappearance time, more marked in those with convulsions. The degree of decrease in the disappearance time varies directly with the degree of severity of the toxemia, increasing with the general clinical improvement.

The same factor, or group of factors, that produces the edema, hypertension, and albuminuria in the late toxemias of pregnancy, apparently produces the condition in the tissues which give a reduced disappearance time. Hence the intradermal salt solution test may prove a valuable aid in diagnosis and prognosis of those conditions.

The use of the test routinely during the later months of pregnancy may prove of value in determining the oncoming of a toxemia earlier than by other methods now available.

THYROID DISEASE: Diagnostic Value of the Kottmann Reaction in Thyroid Dysfunctions, Katayama, I. Am. Jour. Med. Sc., July, 1926, clxxii, No. 1, p. 84.

A study of 101 cases with the conclusions following:

It is generally conceded among physiologists and clinicians that the determination of the basal metabolic rate is the most dependable laboratory index of thyroid activity. Increased thyroid secretion produces a lowering of the tolerance for carbohydrate, but there are numerous other conditions in which the tolerance for carbohydrate may be diminished. Hence the occurrence of high blood and urine sugar curve after the ingestion of glucose is not in itself indicative of hyperthyroidism. In hyperthyroidism, however, the blood and urine sugar curves after glucose furnish information concerning a phase of carbohydrate metabolism which is not gauged by the basal metabolic rate.

The basis of the Kottmann reaction is obscure, and hence it is difficult to say in what manner thyroid activity influences it. Such a reaction can only be accepted with skepticism. From the data reported in this paper it is evident that the results of the Kottmann reaction

are not in accord with the basal metabolism or the glucose tolerance. The retardation of the reduction of the silver iodode to silver occurs in many and various conditions patently not due to hyperthyroidism. Its diagnostic value in detecting hyperfunction or hypofunction of the thyroid is very dubious. It cannot be accepted as a substitute for the determination of the basal metabolic rate or of glucose tolerance.

ECLAMPSIA: The Blood Chemistry in Eclampsia, Stander, H. J., and Radelet, A. H. Bull. Johns Hopkins Hosp., June, 1926, xxxviii, No. 6, p. 423.

Zweifel, in 1904, showed that in the urine of eclamptic women the urea nitrogen is lowered and the ammonia nitrogen raised. From this, he reasoned that there ought to be an increase in some acid in the blood, and that it was probably in lactic acid. He accordingly analyzed the urine and blood for that substance, precipitating it as zinc lactate. He reported eight eclamptic cases in which he was able to show the presence of lactic acid in the blood. From one of the patients, he obtained two specimens of blood, the first showing lactic acid, while the second did not, this second sample of blood having been taken about five hours after the last convulsion. He furthermore stated that in normal pregnancy he was usually unable to demonstrate the presence of lactic acid. It might be added that Futh and Lockemann demonstrated the presence of lactic acid in the cerebrospinal fluid of eclamptic patients. The authors' figures on the other hand, show that, in the blood of normal pregnant women, the lactic acid is within the limits for the normal nonpregnant person, and that in eclampsia there is a pronounced increase, amounting to 200 per cent or more above the normal.

A sample taken from an epileptic pregnant woman a few minutes after a violent convulsion showed only a slight increase in lactic acid, (45.32 mg.) so that the increase is not markedly influenced by the muscular activity due to convulsions.

Neither does it seem to be due to interference with the excretion of lactic acid.

The authors suggest three possibilities to explain the high lactic acid content of the blood: (1) An abnormally great amount of carbohydrate metabolized; (2) a disturbance or slowing down of the "lactic acid to glycogen" step in the carbohydrate chain; (3) lactic acid production from sources other than carbohydrate, such as protein.

ANACIDITY: A Statistical Study of the Diagnostic Value of Anacidity, Hartman, H. R., and Sager, W. W. Med. Jour. and Rec., New York, July 21, 1926, cxxiv, 96.

A study of 492 cases studied by fractional gastric analysis from which it is concluded that the chances for and against the presence of pernicious anemia, gall bladder disease, or carcinoma, in a case of anacidity, are about even; the chances of either pernicious anemia or carcinoma being present are about one in three.

Carcinoma is rare when free hydrochloric acid is present.

PERNICIOUS ANEMIA: The High Color Indices in Pernicious Anemia, Komiya, E. Fol. hematol., May, 1926, xxxii, 201.

Komiya recalls that while the high color index so frequently noted in pernicious anemia has been ascribed to the large number of megalocytes present, this conclusion has been disputed.

He reports his studies in this disease leading to the conclusion that, in pernicious anemia, not only the megalocytes but all the larger erythrocytes, except those showing polychromasia, carry larger amounts of pigment and that the increase of the color index is due to a general hyperchromia of the erythrocytes.

The hemaglobin content of embryonic blood is relatively high, but the erythrocyte count is less than in the adult. As a result the embryonic color index is high due to the presence of abnormally large red cells as shown by a high volume index. The so-called normoblasts of embryonic blood correspond to the macroblasts or macrocytes of adult blood.

Komiya states that in pernicious anemia the blood formation is similar to that in embryonic life and that the increased color index is caused, not only by the megalocytes in Ehrlich's sense, but also by the macrocytes which are an even more important factor.

Laboratory Technic

HOOKWORM: The Place of the Smear in Hookworm Diagnosis, Hausheer, W. C., and Herrick, C. A. Am. Jour. Hyg., July (Supplement), 1926, vi, 136.

The smear method is diagnostically accurate if the intensity of infestation is such that 500 or more ova are present per gram of feces.

The smear method, if only two slides are examined for a negative, will fail in a certain percentage of cases yielding between 300-500 per gram of stool.

If less than 300 ova are present per gram of stool the smear is highly inaccurate.

When the above factors are taken into consideration the smear method may be used with confidence; it is simple, rapid and still holds a valuable place in the fecal examination for hookworms.

HOOKWORM: Evaluation of the Methods of Stoll and Lane in Light Hookworm Infections, and Accuracy in Diagnosis of the Willis Floatation Method, Hausheer, W. C., Herrick, C. A., and Pearse, A. S. Am. Jour. Hyg., July (Supplement), 1926, vi, 118.

In a series of seventy carefully studied stools the regular Stoll technic (large drop) will detect cases having as low as fifty eggs per gram by Lane count, if not less than two slides are examined.

Lane's method, pushed to finality, demonstrated 68.3 per cent of the ova indicated present by the regular drop dilution egg-count, and diagnostically showed every stool positive.

The small drop Stoll technic demonstrated 94.5 per cent of the ova, shown present by the regular dilution counts. The small drops failed diagnostically (two slides only being counted from each tube) in 75 to 80 per cent of cases, having less than 200 ova per gram, but showed high diagnostic efficacy in the higher counts.

An analysis is presented of the Willis floatation method, and its efficacy, when properly handled, is emphasized. It would appear to be as accurate as Lane's direct centrifugal floatation when certain details in its use are observed.

The floatation method, as practiced by the authors, is as follows:

1. The specimen of feces is thoroughly mixed in the container.

2. A small amount (1 to 2 gm.) is *thoroughly* comminuted with salt solution having a specific gravity of 1.150 to 1.200.

Care in the comminution of feces and salt solution, and in the use of a solution of proper density, are essential factors for success.

3. The container is then filled to the brim with additional saline solution.

4. A glass slide, of such size that it will more than cover the container, is placed thereon and allowed to stand for ten to fifteen minutes.

As ova are destroyed by concentrated saline in an hour, this period should not be longer than thirty minutes.

5. Remove the slide carefully without losing the adherent fluid, quickly invert, and examine under low power.

CULTURE MEDIA: The Exudate from Nutrient Agar Slants—The So-Called Water of Condensation, Healy, D. J. Jour. Bacteriol., September, 1926, xii, No. 3, p. 179.

As is well known, a variable quantity of liquid collects in nutrient agar slant tubes, a condition which does not occur in the case of nutrient gelatin slants. For many years this liquid has been known as the water of condensation.

As many microbes grow more freely in this so-called water of condensation than they do on the surface of the agar slant or in nutrient broth, it seemed desirable to determine its composition.

As a result of his analysis the author concluded that the so-called water of condensation which collects in nutrient agar slant tubes is an exudate from the nutrient agar, possessing nutrient substances suitable for sustaining bacterial growth.

HOOKWORM: Estimation of the Number of Hookworms Harbored by the Use of the Dilution Egg Count Method, Hill, R. B. Am. Jour. Hyg., July (Supplement), 1926, vi, 19.

In order to check the suggestion of Stoll that the factor 44 represents the average egg output per gram of feces per female hookworm, and that such a factor can be used to estimate the number of parasites when only eggs per gram are known, the total egg output of 93 heavily infested cases was estimated for two, or three days, and compared with the number of worms recovered after treatment to a cure.

A positive correlation was found between each of the items: the total daily egg output, the number of eggs per gram, basis formed, and the number of female hookworms harbored.

The calculations resulted in finding that the factor 18.3 represented the number of eggs per gram per female in this series. This factor, when applied to the egg counts in the series, gave a close estimate of the number of female hookworms harbored, but when applied to other groups was usually too low. Stoll's proposed factor of 44 was too high.

It was found that feces recovered could be classified as formed, soft, or mushy, and the average amounts of these classes, per day, were 147, 226, and 314 grams respectively, a ratio of approximately 1 : 1.5 : 2. It is suggested that, for comparison, all counts per gram be reduced to the basis of formed stools by the use of the proper factor.

It is suggested that as the number of worms harbored increases, the egg output per worm decreases.

IMMUNITY: The Rôle of the Reticulo-Endothelial System in Immunity. II. The Complement Titer After Blockade and the Physiologic Regeneration of the Reticulo-Endothelial System as Measured by Reduction Tests, Jungeblut, C. W., and Berlot, J. A. Jour. Exper. Med., June, 1926, xliii, No. 6, p. 797.

Intravenous injections of India ink into guinea pigs caused a decided drop in the complement titer which set in as early as fifteen minutes after the injection, but did not reach its maximum for three hours. This drop was followed by a return to normal within the first twenty-four hours following the injection.

India ink mixed in vitro with guinea pig serum adsorbs the complement almost immediately to its full extent.

By means of reduction tests (methylene blue and nitroanthraquinone) it was shown that the respiration of the cells of the liver and spleen of guinea pigs was markedly impaired for the first eight hours, following an intravenous injection of ink. Evidences of a return to normal functional vitality, however, became apparent by the end of the first day after the injection.

GONOCOCCUS: Study of Agglutination of the Gonococcus in Man, Jenkins, C. E. Brit. Med. Jour., July 3, 1926, xi, 3417.

Agglutinins for the gonococcus are not produced in man when the infection is limited to the genitourinary system. In generalized infections, such as arthritis, the production of agglutinin is so slight and uncertain as to render such a test useless for diagnosis.

STAINING TECHNIC: A New Staining Dish, Kracke, R. R. Jour. Am. Med. Assn., July 3, 1926, lxxxvi, 29.

The entire apparatus is constructed in three parts; the support, outside container, and slide rack. The stand is made of rigid black enameled iron with a row of ten microburners which supply an even and adequate distribution of heat to the stain within and is so arranged that the container can be moved forward or backward to facilitate drainage from the outlet petcock on the bottom.

The outer container and slide rack are both constructed of monel metal, which is noncorrosive and extremely durable, insuring long continued usage. The outer container consists of a metal rectangular box of outside dimensions of approximately 1½ by 3

by 10 inches and a capacity of 600 c.c. when empty and 400 c.c. when filled with slides. At one end near the top is an inlet to which a water hose can be connected, and at the opposite end on the bottom an outlet petcock through which the stain and water can be drained. The bore of the inlet and outlet is of sufficient caliber to insure a rapid inflow and a rapid outflow of stains or reagents, the dish emptying in about twenty seconds with the petcock open fully.

The slide holder fits snugly into the outer container, with sufficient space allowed so that it can be lifted and replaced easily, facilitating the thorough washing of slides by repeated immersion. It consists of a framework containing fifty slots for that number of slides and a bottom slide rest, the construction being such that the rack filled to its full capacity can be vigorously shaken to remove excess water or stain.

Technic for use: The required number of specimens to be stained are placed within the rack and then placed into the outer container. The first stain to be used is then poured into the end space provided for that purpose, the stain flowing beneath the slides and welling up between them. Sufficient stain is poured in to cover the slides to the highest level of the smears, the upper portion of the slides remaining free from stain.

After the stain has acted for the required time, it is drained back into the stock bottle through the lower petcock. The other stains and reagents are poured in and drained off in a similar manner. Whenever water is indicated in the process it is allowed to flow in through the upper petcock and drained off; or a continuous flow can be maintained when thorough washing is desired.

After completion of the staining process with tissue sections, they can be removed one by one and mounted; or, if suspected tuberculous sputum is being stained, the entire rack can be removed and the slides allowed to dry in the air. When staining tuberculous sputum, the same procedure is carried out, the necessary heat for steaming with carbol-fuchsin being provided by the burners beneath.

TUBERCULOSIS: A Method of Producing "Defatted" (Nonacid-Proof), Living Cultures of B. Tuberculosis with a Preliminary Report on the Same as an Immunizing Agent, Whitman, R. C., and Chambers, K. L. Colorado Med., April, 1926, xxiii, 118.

The method depends upon the use of the following culture medium:

The ends of long bones or the vertebra of cattle, rich in marrow, are stripped as clean as possible of fat, tendon, ligament, and muscle, and broken up with a hammer and chisel into pieces the size of an English walnut, or better, ground in a green bone grinder, such as is used by poultry men. It is not necessary to exclude rigidly portions of the bone from the shaft side of the epiphyses, but the red marrow gives better results than the yellow marrow, and should make up as much of the material as possible. Weigh, place in a suitable vessel, and boil over the open flame for an hour or more. Decant the fluid, and when cold, remove the thick layer of fat which collects at the surface. Add water to make the amount equal in cubic centimeters to the weight of the bone in grams. Add 0.5 per cent salt, 1 per cent Witte peptone, and 1.5 to 2 per cent powdered agar, heat to dissolve these, and without filtering, tube and autoclave. All the lots we have so far made up have been neutral or very slightly acid to litmus without adjustment. As the tubercle bacillus does best on media somewhat more acid than that best adapted for general purposes, the reaction above mentioned serves very well.

On this medium the tubercle bacillus is nonacid-fast, the property being developed after transfer to egg media or media containing lecithin.

A small series of guinea pigs was immunized with such cultures and later inoculated with, the authors believe, encouraging results.

Their conclusions follow: On agar medium made from bone instead of meat infusion, the tubercle bacillus grows wax free; (i.e., nonacid-proof), in the first generation.

Vaccines prepared by the customary method from such wax free cultures of an old, greatly attenuated strain of human type bacillus, afford practically complete protection to guinea pigs against subsequent injection of overwhelming doses of virulent bovine bacilli.

This protection lasts for about one year, and no doubt for a longer time, though in somewhat lower titer.

The same vaccine seems to have considerable curative value in established tuberculosis.

The wax free organism may prove to be a useful antigen for complement-fixation tests.

It is to be hoped that improvement in the method of preparing and using the vaccine may increase its usefulness. Possibly the use of living wax free bacilli as a vaccine may be found to be both safe and advantageous.

BLOOD CALCIUM: A Colorimetric Method for the Estimation of Blood Calcium, Roe, J. H., and Kahn, B. S. Jour. Biol. Chem., March, 1926, lxvii, 585.

Reagents:

Trichloracetic acid 20 per cent.
Phenolphthalein 1 per cent.
Sodium hydroxide, calcium free 20 per cent.
Trisodium phosphate 1 per cent.
Sulphuric acid 5 per cent.
Sodium or ammonium molybdate 5 per cent.

Standard phosphate solution: Dissolve 4.394 gm. of pure dry monopotassium phosphate in 1,000 c.c. of water. One c.c. contains 1 mg. of phosphorus. Preserve this stock solution with chloroform.

Five c.c. of this solution diluted to 1,000 c.c. with phosphate-free water is the solution for calcium estimation, 10 c.c. containing 0.05 mg. of phosphorus equivalent to 0.097 mg. of calcium phosphate.

Hydroquinone-bisulphite reagent: 30 gm. sodium bisulphite and 1 gm. hydroquinone (highest purity), dissolved in 200 c.c. phosphate-free water. Calcium-free filter paper.

Place 2 c.c. blood serum in a small flask and add 4 c.c. of distilled water and 4 c.c. of 20 per cent trichloracetic acid. Mix thoroughly, allow to stand ten minutes, and filter through a double acid-washed calcium-free filter paper. Transfer 5 c.c. of the trichloracetic filtrate to a 15 c.c. conical centrifuge tube which has been thoroughly cleaned by immersion in bichromate sulphuric "cleaning solution" for several hours. Place one drop of 1 per cent phenolphthalein in the tube and add, a drop at a time, 20 per cent calcium-free hydroxide until a definite pink color is obtained. Add 1 c.c. of 1 per cent trisodium phosphate, twirl the tube until thoroughly mixed, cork, and set aside for one hour.

After one hour's standing, centrifuge for three minutes. Decant carefully the supernatant fluid from the calcium phosphate precipitate. Place the inverted tube upon a pad of filter paper to drain for two or three minutes, then wipe away adherent solution from the mouth of the tube with a clean cloth or paper. Wash twice with 5 c.c. portions of 50 per cent alcohol made faintly alkaline to phenolphthalein with a few drops of calcium-free alkali. In washing, the mat of calcium phosphate in the bottom of the tube must be thoroughly broken up with a glass stirring rod, and the process of centrifuging, decanting, and draining the tube should be carried out as described above. Dissolve the washed precipitate in 5 c.c. of 5 per cent sulphuric acid by volume (5 c.c. concentrated acid per 100 c.c. of water), and decant into a Rothberg-Evans sugar tube, or a graduated test tube; wash the centrifuge tube twice with approximately 3 c.c. and 2 c.c. portions of the 5 per cent sulphuric acid, adding the washings to the graduated tube.

In a similarly graduated tube place 10 c.c. of standard phosphate solution containing 0.05 mg. of phosphorus, and add 0.5 c.c. of concentrated sulphuric acid. Now add to each tube 1 c.c. of 5 per cent sodium molybdate and 1 c.c. of hydroquinone bisulphite reagent. Place the tubes in a boiling water-bath for ten minutes. Remove, cool, dilute the standard to 15 c.c. and the unknown to a volume giving a color that will approximately match the standard (15 c.c. in normal bloods), and compare in a colorimeter in the usual manner.

The calcium content in milligrams per 100 c.c. of serum is then calculated from the formula $\frac{R_1}{R_2} \times 0.05 \times \frac{D_2}{D_1} \times \frac{60}{31} \times 100$ in which R_1 is the reading of the standard, R_2 the reading of the unknown, D_1 the dilution of the standard, and D_2 the dilution of the unknown.

The Journal of Laboratory and Clinical Medicine

VOL. XII ST. LOUIS, MO., APRIL, 1927 NO. 7

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Official Organ of the American Society of Clinical Pathologists

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EDITORIALS

New Views on Carbohydrate Metabolism

THE researches of the past few years dealing with the chemistry of muscular contraction have led to the conclusion that carbohydrate and this alone is oxidized in the process. The evidence for this belief is mainly that the volume of CO_2 produced during the entire process of contraction and restoration to the original state is equal to that of the oxygen consumed; the respiratory quotient is unity. This has been shown both for isolated muscle contracting outside the body and for the extra amounts of O_2 and CO_2 used by the intact animal (man) while performing muscular work. To obtain the latter values the resting (basal) amounts of O_2 used and of CO_2 expired by the animal are subtracted from the amounts used and expired not only during the exercise itself but also for such a period thereafter as is necessary to bring the values back to the basal level. Supporting evidence for the same view is also afforded by the fact that direct chemical analysis of the muscle reveals dimin-

ution in the amount of glycogen as a result of contraction and no significant change in any other organic constituent.

If we accept this view it leads to the very important conclusion that proteins and fats can be used for the production of muscular energy only after they have been converted into carbohydrate. That such conversion readily occurs in the case of protein is well known as a result of studies of the metabolism in diabetes, but the same studies are usually considered also to show that no carbohydrate is derived from fat or, more correctly, from fatty acid since the glycerol portion of fat itself is readily changed into sugar. The main evidence for this belief is that the ratio between the excretion of dextrose and nitrogen in a starving, or protein-fed, animal poisoned with phlorizin remains at a constant level (1:3.65) from day to day. But such an animal can scarcely be regarded as strictly a diabetic one since at least two of the cardinal symptoms of this disease are missing, namely, hyperglycemia and marked ketosis. When depancreatized animals are observed, on the other hand, the D.N. ratio does not, as a rule, remain constant from day to day, whereas all the symptoms of the diabetic state as observed in man are prominent. This type of evidence against the conversion of fat to carbohydrate is, therefore, inconclusive and when we remember that conversion of fat to carbohydrate can easily be shown to occur in plants and that the process is quite explicable on a purely chemical basis, the probability that fat is converted to carbohydrate in the metabolism of all animals becomes considerable.

To convert protein and fat into carbohydrate requires that more oxygen be incorporated in the molecule since the former is built up of numerous methyl (CH_3) groups and the latter of alcohol (CHOH) groups. This absorption of oxygen must consequently lead to very marked decrease in the R.Q. ($\frac{CO_2}{O_2}$). Thus, if we compare the chemical formulae of fatty acids found present in the animal body with that of a simple sugar it is clear that the quotient must fall to about 0.2 or 0.3. But actual analysis of the respired air of completely diabetic animals, in which it is believed this new formation of sugar will be proceeding at its fullest intensity, shows that the quotient is never lower than 0.66. How are we to explain this? We can do so if we assume that the oxygen absorption occurs somewhere in the body outside the muscles, the partially oxidized molecule being then carried as sugar to the muscles where it is oxidized. The R.Q. of the animal as a whole will then be the algebraic sum of the low quotient of the gluconeogenic process occurring in the liver and the high one of the final oxidation process occurring in the muscles. That this *net* quotient should under certain standardized conditions remain at a constant level, as in a phlorizin-poisoned dog, is no more remarkable than that the body temperature, or the blood sugar or the daily excretion of nitrogen remains constant.

According to such a view the main fault in metabolism responsible for diabetes must be excessive gluconeogenesis. This is admitted by all investigators to be the case for protein, but is denied for fat. And yet there is much to indicate that fat is also concerned. Fat metabolism certainly goes wrong in diabetes and it has been suggested that the ketone bodies are really by-

products of the chemical process by which fat is converted into carbohydrate. Attempts to disprove this hypothesis, by seeing whether feeding with fats will increase the sugar excretion in diabetes, are of course futile, since it is well known that fat after its absorption goes through a lengthy process before it is finally drafted to the liver to be prepared for ultimate oxidation. Experience, both in the laboratory and the clinic, affords strong support to the "fat derivation" hypothesis. Thus, we have observed that when depancreatized dogs treated with insulin are made fat, by feeding them with excess of carbohydrate, they exhibit much more acute symptoms of diabetes when insulin is withdrawn than are observed under the same circumstances in the case of thin dogs. The hyperglycemia, ketonemia and glycosuria are all more intense, but most striking of all, the general symptoms are extremely acute and a fat animal seldom lives for more than four days after discontinuing the insulin, whereas a thin one may live several weeks.

There is, therefore, no irrefutable evidence against the view that fat, as well as protein, must be converted into carbohydrate in the liver before it can be utilized as fuel by the muscles. On the contrary, recent results are all on its support and among these, experiments by Soskin may be of interest. It is well known that the blood sugar steadily falls when the liver is removed from the body. This has been considered to show that the liver must at least be the chief source of the blood sugar, but it has not been concluded that it is its only source. Soskin injected large amounts of epinephrin into hepatectomized dogs and subjected others to asphyxia under ether anesthesia without causing even the slightest increase in the steadily falling blood sugar, and he found after death that the muscles still contained some glycogen. The glycogen of muscle cannot apparently be reconverted to sugar in the body. It can of course be thus converted in vitro by hydrolysis, either with acid or diastase, but once it has become deposited within the living muscle it has entered an irreversible reaction which leads it through lactic acid to CO_2 and H_2O. In asphyxia, as in Soskin's experiments, no doubt so much of this lactic acid accumulated in the muscles that some found its way into the blood. If the liver had been present this blood-lactic acid would have been converted into glucose, for this process is known to occur in the intact animal, but in the liverless animal it remained unchanged, and there was no rise in blood sugar. That the glycogen in the muscles should diminish, as Mann and Magath have shown, in the liverless animals is of course easily accounted for by the constant using up of this material.

It may be pointed out that Markowitz has found that the respiratory quotient rises sometimes almost to unity for periods of several hours' duration after removal of the liver in dogs which are kept alive by injection of sugar. It does not rise so high without sugar injection but that it rises at all, over sufficiently long periods of time to rule out any error due to a blowing off of CO_2 is supporting evidence for the above views.

Finally it may be concluded that an important function of insulin consists in its diminishing, or inhibiting, the overactive gluconeogenesis which is the cause for the excessive sugar production by the liver. Linked closely with this process, is that of glycogen formation in the liver (glucogenesis) for

which the presence of insulin is also essential, although available evidence indicates that glycogen formation in the muscles can proceed in the absence of insulin.

—*J. J. R. M.*

Progress in the Treatment of Pernicious Anemia

EVERY clinician realizes how unsatisfactory has been the treatment of pernicious anemia. Many different drugs and methods of treatment have been suggested. Only hydrochloric acid in large doses, arsenic and transfusion have stood the test of time as therapeutic measures of proved values. These, however, have only aided in initiating or prolonging the remissions. Nothing has materially altered the inevitably fatal course of the disease.

Most observers agree that true pernicious anemia is of intestinal origin. Numerous workers, beginning with Herter, have thought that the type of intestinal flora present may be the determining factor in the production of poisons with an affinity for the hematopoietic and nervous systems. The proof offered for this view is not convincing. The same may be said for the theory that certain yeasts may be the causative agent.

Clinicians have emphasized that patients suffering from pernicious anemia have often taken for a long time an incomplete diet. Bohan[1] has especially called attention to the fact that there is commonly a protein deficiency over a long period. Other patients may give a history of taking excess fats. It is also possible that toxic bodies may be formed as the result of altered digestion consequent upon the deficiency of hydrochloric acid in the stomach, a constant finding in the disease.

Koessler, Mauer and Laughlin[2] think that changes in the viability and permeability of the intestinal wall may be important factors. Certain products of normal digestion are toxic if introduced into the blood stream. Normally the intestinal wall is an effectual barrier to the entrance of poisons into the blood. McGarrison has pointed out the very striking changes which take place in the intestinal wall as the result of vitamin deficiency. Koessler, Mauer and Laughlin produced a marked anemia in the rat by feeding a vitamin deficient diet, which they think is due to altered permeability of the intestinal wall. They state that suggestive good results have been obtained in patients with pernicious anemia by feeding a vitamin rich diet.

Certainly the most promising results so far obtained from any method of treatment of pernicious anemia are those reported recently by Minot and Murphy.[3] They have treated a large series of patients with a full protein diet including at least a half pound of liver a day. Fats have been much restricted, and fruits and vegetables supplied in abundance, but liver is seemingly the most important item in the diet. The use of liver is thoroughly justified from experimental work, since Whipple and Robscheit-Robbins[4] found that it is especially valuable in hastening blood regeneration in dogs with experimental anemia.

It is as yet not clear whether the favorable action is due to the fact that liver is a complete protein or contains some specific antihemolytic factor.

Conclusions must certainly be cautiously drawn since pernicious anemia often runs a bizarre course with frequent spontaneous remissions. Clinicians elsewhere are, however, experiencing equally as good results as those reported by Minot and Murphy by the proper use of the diet suggested by the Boston investigators. There is uniformly a rise in the reticulocytes and a fall in the icterus index indicating there is more active regeneration and less active destruction of red cells with the liver diet.

The introduction of this diet by Minot and Murphy marks a distinct advance in the treatment of pernicious anemia.

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—*R. L. H.*

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ON TO WASHINGTON

American Society of Clinical Pathologists

THE eyes of the American Society of Clinical Pathologists are turned towards the National Capital where our members are due to convene in annual session in the Springtime. The date is set for May 13, 14, and 16, 1927, and the place of meeting is the Willard Hotel. The first two days, Friday and Saturday, will be devoted to the scientific program. Monday there will be a business session. Ample opportunities are, therefore, afforded the members for fraternization and exchange of thought, as well as for sight seeing. The Sixth Annual Convention of the American Society of Clinical Pathologists is looked forward to with great interest by all of the members not alone for the scientific program but also because questions of momentous importance will be discussed at our business session, problems fraught with the continued economic existence of our specialty.

The scientific papers, as will be seen from the accompanying program, are of unusual interest and will undoubtedly bring forth a liberal discussion. They cover a comprehensive field in the domain of Serology, Hematology, and Tissue Pathology as well as other items that are of everyday importance in the function of the clinical pathologist. A very useful feature will be the exhibits, both scientific and commercial, which is growing in importance with every year.

A most enjoyable item on the program will be the annual banquet, which aside from its gastronomic and social features bids fair to outdo all previous efforts by the galaxy of eminent speakers who will grace the festive board. These include, Dr. George K. Burgess, Director of the Bureau

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of Standards, President William G. Exton, Dr. M. T. MacEachern of the American College of Surgeons, and Dr. George W. McCoy, Director of the United States Hygienic Laboratories.

During the six years of the existence of the Society an organization has been built up which has done valued service to the cause of scientific medicine both in private and hospital practice. Closely cooperating with the American College of Surgeons the laboratories in all standardized hospitals have, through the efforts of the American Society of Clinical Pathologists, been placed on a high level of efficiency. The clinical pathologists occupying the directorships of these institutions have been a power and a force in stimulating the attending staffs to the adoption of exact methods in diagnosis and therapy. The laity too has become educated to the important rôle that the pathologist plays in the teamwork of the hospital and his active collaboration with the clinician.

The date of the meeting has been purposely placed as near to the American Medical Association Convention as possible in order to give our members opportunity to attend the big gathering. They will also be able to take advantage of the reduced transportation rates. By carrying the program over from the week-end to Monday, the intervening Sunday can be profitably devoted to committee meetings, also the pleasant reunions among comrades in the common cause, the swapping of reminiscences of our early struggles, in the formation of new friendships and pleasant associations. The wives, too, and members of families of the Fellows will find in the convention a great opportunity both for sight seeing and social enjoyment. The local committee in Washington is making plans for their entertainment.

The business session promises to be extremely interesting to all the members and they are asked to participate actively therein. It will touch on questions that vitally affect their future. The first topic of discussion will probably center on the report of the State Laboratory Committee. The recent questionnaire sent out to the members has provoked considerable thought and aroused an enthusiastic response. The Committees on Research, Publication, and Registration of Technicians will also present reports which will arouse liberal discussion. It is incumbent on each member to immediately make arrangements to attend the convention. The time spent will be more than compensated for by the stimulation received in listening to the scientific papers and by contact with colleagues in the same planes of activity. He will not only receive information that will be useful to him in his daily practice but will confer the same benefit on his fellow members. It must be remembered that only by combined effort has it been possible to place our specialty on the high plane that it is occupying at present in the field of medicine equal to that of other branches. Your attendance at the Convention will lend encouragement to your fellow workers in the field of clinical pathology.

Judging from the response to our inquiry as to prospective attendance, the Sixth Annual Convention of the American Society of Clinical Pathologists promises to eclipse all previous records.

DR. WARD BURDICK
Denver, Colo.
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Tampa, Fla.
Member of Board of Censors

American Society of Clinical Pathologists Sixth Annual Convention

WILLARD HOTEL, WASHINGTON, D. C.

FRIDAY, MAY 13, 9 A.M.

CALL TO ORDER

SHORT BUSINESS SESSION

Scientific Program

Symposium on Kahn Test.

A Study of the Micro-Kahn Test in Syphilis. By Robert A. Kilduffe, M.D., Atlantic City, New Jersey.

The Microscopic Kahn Reaction. By Francis B. Johnson, M.D., Charleston, South Carolina.

Further Studies of the Kolmer and Kahn Tests. By C. E. Roderick, M.D., Battle Creek, Michigan.

Discussion opened by Dr. R. L. Kahn, Lansing, Michigan.

Fatalities Following the Use of Arsphenamine with Report of Autopsy. By Ernest Scott, M.D., and R. A. Moore, M.D., Columbus, Ohio. (Read by title.)

Brain Structure Changes After Treatment in General Paralysis. By A. M. P. Saunders, M.D., Dunning, Illinois.

The Use of Injection Methods in Pathology. By Ernest Scott, M.D., and R. A. Moore, M.D., Columbus, Ohio.

FRIDAY, MAY 13, 2 P.M.

The Blood Picture of Purpura. By Nathan Rosenthal, M.D., New York City.

Anemia as a Factor in the Test of the Rate of Sedimentation of the Erythrocytes. By Roger S. Hubbard, Clifton Springs, New York (By invitation).

Studies of Sedimentation of Erythrocytes. By A. H. Sanford, M.D., I. Technic, M.D., and H. F. Hunt, M.D., Rochester, Minn.

Differential Blood Counts: A Comparison of the Accurary Obtained by Various Methods. By Dean N. Beacom, M.D., Denver, Colo.

Ovarian Function: Its Influence on the Concentration of Calcium in Blood. By Herman Sharlit, M.D., and Wm. G. Lyle, M.D., New York City.

Purpuric Smallpox, Review of Recent Studies. By Kano Ikeda, M.D., St. Paul, Minnesota.

FRIDAY, MAY 13, 8 P.M.

A Key to the Diagnosis of Neoplasmata. By Dr. Wm. Carpenter MacCarty, M.D., Rochester, Minnesota.

Rapid Methods of Examining Tissue Microscopically Without a Microtome. By B. T. Terry, M.D., Rochester, Minnesota.

The Present State of Our Knowledge of Gingivitis. By Robert A. Keilty, M.D., Danville, Pennsylvania.

The Etiologic and Specific Relationship of Foci of Infection to Certain Organic Lesions: A Postmortem Study. By A. S. Giordano, M.D., South Bend, Indiana.

The Value of Tonsil Cultures in Cases of Focal Infection. By Russell Richardson, M.D., Philadelphia, Pennsylvania.

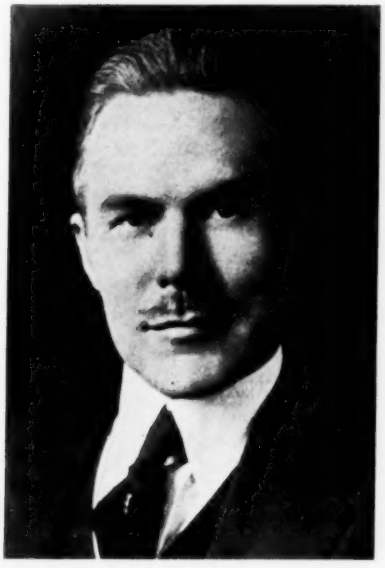
SATURDAY, MAY 14, 9 A.M.

Accuracy and Precision in Clinical Pathology. By P. V. Wells, Dr.Sc., Newark, New Jersey (by invitation).

New Clinical Methods for Measuring Color and Turbidity as Applied in the Junior Scopometer. By Wm. G. Exton, M.D., Newark, New Jersey.

A Turbidimetric Method for Sugar. By Anton R. Rose, Ph.D., Newark, New Jersey. (By invitation.)

DR. PHILIP HILLKOWITZ
Denver, Colo.
Chairman, Executive Committee



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Philadelphia, Pa.
Member of Executive Committee
Chairman, Publication Committee

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Detroit, Mich.
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DR. HERMAN SPITZ
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DR. FREDERIC E. SONDERN
New York City
Chairman, Public Relations Committee

DR. REUBEN OTTENBERG
New York City
Chairman, Program Committee

DR. GEORGE K. BURGESS
Washington, D. C.
Director, Bureau of Standards
Speaker at Annual Banquet

DR. M. T. MACEACHERN
Chicago, Ill.
Associate Director, American College of Surgeons and Director of Hospital Activities
Speaker at Annual Banquet

Sugars in Normal Urine. By Isadore Greenwald, M.D., New York City. (By invitation.)
Occurrence of Lipoids in Urine and Their Diagnostic Importance. By E. L. Miloslavich, M.D., Milwaukee, Wisconsin.
The Comparative Diagnostic Value of the Levinson Test and the Glucose Content of the Cerebrospinal Fluid in Tuberculous Meningitis. By A. S. Giordano, M.D., South Bend, Indiana.

SATURDAY, MAY 14, 2 P.M.

Pathology of Intestinal Tuberculosis. By Alfred Blumberg, M.D., Oteen, N. C.
The Cultivation of Tubercle Bacilli. By H. J. Corper, M.D., and Nao Uyei, Ph.D., Denver, Colorado.
Some Observations on Basal Metabolism. By Leon S. Lippincott, M.D., Vicksburg, Miss.
Pathological Laboratory Examinations for the Dentist. By Charles G. Darlington, M.D., New York City.
Laboratory Examinations Necessary and Unnecessary. By George L. Schadt, M.D., Springfield, Massachusetts.
A Modification of the Technic of the Wassermann Test. By L. H. Cornwall, M.D., D. Groszberg, and Blanche C. Taylor, New York City.

SATURDAY, MAY 14, 7 P.M.

ANNUAL BANQUET

The Relation of Clinical Pathology to Preclinical Medicine. By President William G. Exton, Newark, New Jersey.
The Relation and Responsibilities of the Clinical Pathologist to the Hospital Standardization Movement. By Dr. M. T. MacEachern, American College of Surgeons, Chicago, Ill.
Remarks by Dr. Norris Fishbein, Editor, Journal American Medical Association, Chicago, Ill.
Remarks by Dr. George K. Burgess, Director, Bureau of Standards, Washington, D. C.
Remarks by Dr. George S. McCoy, Director, Hygienic Laboratory, Washington, D. C.

MONDAY, MAY 15, 1927, 9-12 A.M. and 2-5 P.M.

BUSINESS SESSION

Call to order
Reading of Minutes
Unfinished Business
Reports of Committees
- Committee on Exhibits
- Committee on Public Relations
- Publication Committee
- Program Committee
- Research Committee
- Service Bureau Committee
- State Laboratory Committee
- Committee on Registry of Technicians

Election of Members
New Business
Report of Nominating Committee
Election of Officers
Selection of next meeting place
Adjournment

A cordial invitation has been extended to the members of this Society by the Bureau of Standards to visit their Department.